Universidade de Lisboa

Faculdade de Farmácia

Synthesis of a boronic acid-based diazo acetate derivative

Catarina de Oliveira Belmonte Silvério

Mestrado Integrado em Ciências Farmacêuticas

2019

Universidade de Lisboa
Faculdade de Farmácia

Synthesis of a boronic acid-based diazo acetate derivative

Catarina de Oliveira Belmonte Silvério

Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

Orientador: Doutor Pedro Miguel Pimenta Góis, Professor Auxiliar com Agregação

2019

Resumo

Recentemente a investigação em química biológica relativa à descoberta de novos fármacos tem aumentado. Apesar de estes serem necessários, também é preciso identificar novos alvos terapêuticos a nível molecular. Existem vários potenciais alvos ainda por caracterizar, pelo que surgiu a necessidade de desenvolver novas tecnologias para os identificar e explorar as suas funções, tais como as sondas moleculares. Estas consistem em pequenas moléculas concebidas especificamente para se ligarem a um alvo molecular, para estabelecer o papel e os mecanismos de biomoléculas como as proteínas. As moléculas contendo grupos funcionais diazo são uma classe de moléculas que contêm um grupo $-N_2$ ligado a um substituinte contendo carbono. Quando irradiados com luz, do comprimento de onda adequado, podem fragmentar-se em nitrogénio molecular e num carbeno. Este pode ser utilizado para fazer marcação de alvos moleculares. Os ácidos borónicos são uma classe de compostos que contêm um átomo de boro trivalente que possui dois grupos hidroxilo e um substituinte contendo carbono. Estes compostos são conhecidos por formarem complexos reversíveis com compostos hidroxilados em condições fisiológicas, alternando entre uma estrutura trigonal planar sp^2 e uma tetraédrica sp^3. Esta propriedade permite utilizar estes compostos para efetuar o reconhecimento de alvos específicos, como proteínas ricas em serinas e hidratos de carbono.

O objetivo deste trabalho era sintetizar uma nova molécula pertencente à classe dos α-diazocarbonilos com bom rendimento, levando à síntese do 2-diazo-N-(2-(4,4,5,5-tetrametil-1,3,2-dioxaborolan-2-il)fenil)acetamida (**composto 5**). Após concluída a síntese da molécula, o objectivo era testar a possibilidade de ser usada como sonda de fotoafinidade. Infelizmente, não foi possível concluir este objectivo. Proteínas ricas em serinas são uma possível aplicação desta sonda. Estas proteínas são expressas à superfície de várias bactérias *Gram* positivas, e foi provado que possuem um papel vital na adesão destes patogénicos aos tecidos e no desenvolvimento de doença invasiva. Tecnologia que tivesse a capacidade de reconhecer estas proteínas teria um grande valor clínico.

Palavras-chave: Ácido borónico; diazo; sonda; proteína

Abstract

Recently there has been an increased investigative effort in chemical biology towards drug discovery. Not only new medicines are necessary but also new molecular targets. There are innumerous uncharacterized potential targets, from which stems the need to develop new tools that can identify and explore their functions, such as chemical probes. These are small molecules designed to bind tightly to a specific target, to help elucidate the roles and mechanisms of biomolecules, such as proteins. Diazo compounds are a class of molecules that have a $-N_2$ group linked to a carbon-based substituent. When irradiated with light, of the appropriate wavelength, they can fragment into molecular nitrogen and a carbene. This carbene can be used to label molecular targets. Boronic acids are organic compounds characterized by having a trivalent boron atom, which possesses two hydroxyl groups and one carbon-based substituent. In fact, they are known to form reversible complexes with hydroxylated compounds under physiological conditions, shifting from a trigonal planar sp^2 structure to a tetrahedral sp^3 one. This property allows these compounds to be utilized as a recognition moiety for hydroxylated compounds such as serine-rich proteins and carbohydrates.

The objective of this work was to synthesize a novel α-diazocarbonyl molecule within good yield, that could combine the characteristics of both classes of compounds, leading to the synthesis of 2-diazo-N-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide (**compound 5**). After synthesizing the molecule, the goal was to verify if it could be used as a photoaffinity probe. Unfortunately, it was not possible to proceed with this objective. Serine-rich repeat proteins are a possible application of this probe. These proteins are expressed at the surface of many Gram-positive pathogens and have been shown to contribute significantly to the adhesion of the bacteria to the tissue and to the development of invasive disease. A tool that could recognize them would have great value in clinical and analytical practice.

Keywords: Boronic acid; diazo; probe; protein

Agradecimentos

Existem várias pessoas às quais, finda esta etapa, gostaria de agradecer. Se a memória me falhar e me esquecer de alguém peço, desde já, as mais sinceras desculpas.

Ao professor Pedro Góis por me ter recebido no seu laboratório, não só para a realização desta tese mas também para os projectos anteriores desenvolvidos no âmbito do Mestrado Integrado em Ciências Farmacêuticas.

Ao Roberto, pelo tempo e dedicação investidos ao longo destes dois anos na minha formação e pela eterna paciência para com as minhas questões intermináveis e atrasos crónicos.

A todo o grupo de investigação em Química Bioorgânica por me ajudarem sempre que possível.

À minha família e amigos, por me terem ajudado a tornar a pessoa que sou hoje, em especial à minha mãe por todo o esforço ao longo destes seis anos para que o curso visse o seu fim. Ao meu irmão, por ser quem é. Ao Alexandre, por tudo.

Ao Prédio da Maria, por me ter acolhido sem hesitação desde o primeiro dia e por todo o afecto, companheirismo e disponibilidade ao longo destes anos.

À Biblioteca da Faculdade de Farmácia da Universidade de Lisboa, pela oportunidade concedida.

Ao Vitor, por todo o apoio, paciência inesgotável e sentido de humor sempre pronto.

Às minhas colegas Rita Tiago, Marta Esteves, Marta Ribas, Rita Ferreira, Catarina Mendonça, Maria Teresa e Tânia Ribeiro por toda a ajuda e companheirismo ao longo destes seis anos.

Subject Index

1 Introduction 12
1.1 Diazo compounds in chemical biology 12
1.1.1 General Properties 12
1.1.2 Occurrence of diazo groups 12
1.1.3 Diazo compounds as molecular probes 14
1.1.4 Synthetic methodologies 16
1.2 Boronic Acids 22
1.2.1 Structure and Properties 22
1.2.2 Role of boronic acids in chemistry 24
2 Rationale and Goals 29
3 Materials and Methods 31
3.1 General Remarks 31
3.2 Methods 31
3.2.1 Synthetic Pathway A 31
3.2.2 Synthetic Pathway B 37
4 Results 42
5 Discussion 45
6 Conclusions 49
Bibliographic References 50
Annexes 61
A1. ^{1}H-NMR spectrum of compound 5 61
A2. ^{13}C-NMR spectrum of compound 5 62
A3. FT-IR spectrum of compound 5 63

List of Abbreviations

δ: chemical shift

ACN: acetonitrile

BA: boronic acid

BNCT: boronic neutron capture therapy

ºC: celsius

$CDCl_3$: deuterated chloroform

cm: centimeter

^{13}C-NMR: carbon-13 nuclear magnetic resonance

d: doublet

DBU: 1,8-diazabicyclo(5.4.0)undec-7-ene

DCM: dichloromethane

DIPEA: N,N-diisopropylethylamine

DMAP: 4-Dimethylaminopyridine

DMF: dimethylformamide

DMSO: dimethylsulfoxide

DON: 6-diazo-5-oxonorleucine

DONV: 5-diazo-4-oxonorvaline

eq: equivalent

FT-IR: Fourier-transform infrared spectroscopy

g: gram

^{1}H-NMR: proton nuclear magnetic resonance

Hz: hertz

J: coupling constant

M: molarity

m: multiplet

mL: milliliter

mmol: milimol

MRI: magnetic resonance imaging

p-NBSA: *p*-nitrobenzenesulfonyl azide

ppm: parts per million

ROS: reactive oxygen species

q: quartet

rt: room temperature

s: singlet

SRRP: serine-rich repeat protein

t: triplet

TMG: 1,1,3,3-Tetramethylguanidine

THF: tetrahydrofuran

TLC: thin layer cromatography

µL: microliter

UV: ultraviolet

List of Figures

Figure 1: General resonance structures of diazo compounds.

Figure 2: Natural molecules containing diazo groups.

Figure 3: Amino acids containing diazo groups.

Figure 4: General structure of boronic acids.

Figure 5: Examples of boronic acid-containing pharmaceutical agents.

Figure 6: Boronic acid sensor for amyloid β-plaques.

Figure 7: Molecular structure of the desired compound.

List of Schemes

Scheme 1: A-Carbene formation from a diazo compound; B-Insertion reaction from a carbene. C-Wolff rearrangement from a carbene.

Scheme 2: Methods more widely used for the synthesis of diazocarbonyl compounds.

Scheme 3: Regitz diazo transfer method.

Scheme 4: Danheiser modification of the Regitz's method.

Scheme 5: Taber diazo-transfer method with pre-benzoylation.

Scheme 6: Taber strategy modification.

Scheme 7: Diazo transfer method using succinimidyl diazoacetate.

Scheme 8: General reaction of hydrazone dehydrogenation.

Scheme 9: General method to the synthesis of diazo compounds via Swern's reagent.

Scheme 10: Bamford-Stevens reaction.

Scheme 11: House modification of the Bamford-Stevens reaction.

Scheme 12: Fukuyama method to obtain diazocarbonyl compounds.

Scheme 13: Forster reaction.

Scheme 14: Equilibrium between boron sp2 and sp3 structures under physiological conditions.

Scheme 15: A-General mechanism of action of proteolytic enzymes in the active site. B-General mechanism of enzyme inhibition mediated by boronic acids.

Scheme 16: Synthetic scheme of pathway A.

Scheme 17: Synthetic scheme of pathway B.

Scheme 18: Synthesis of N-N'-ditosylhydrazine.

Scheme 19: Step 1 of synthetic pathway B.

Scheme 20: Step 2 of synthetic pathway B.

List of Tables

Table 1: Effect of different bases and solvents on the last step of pathway A.

Table 2: Effect of different bases and reaction times on step 1 of pathway B.

Table 3: Effect of different bases and reaction times on step 2 of pathway B.

1 Introduction

1.1 Diazo compounds in chemical biology

1.1.1 General Properties

Diazo compounds are a class of compound characterized by having a -N_2 group (=N^+=N^-) linked to a carbon atom and they can be aliphatic, aromatic or heterocyclic. They are usually very reactive and are considered important reagents due to their capacity to take part in a series of reactions including 1,3-dipolar cycloadditions, carbene insertions and alkylations.[1,2]

The simplest diazo compound is diazomethane and it was discovered by Pechmann in 1984. This compound presents itself like a yellow gas and it is extremely poisonous[3,4] and carcinogenic.[1] Nevertheless it is very useful in chemical synthesis for example, in the formation of methyl ethers from phenols and alcohols, in cyclopropanation reactions[5] and to this day is still commonly used as a reagent in synthetic organic chemistry.[6]

Diazo compounds are delicate to handle due to their explosive properties, which come from the rapid conversion between resonance structures (**Figure 1**).[7] Recent methodology advances make diazo compounds easily accessible, allowing also the synthesis of biocompatible diazo derivatives. This makes them suitable for several applications in chemical biology.[6]

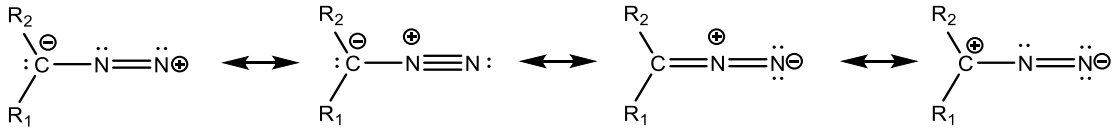


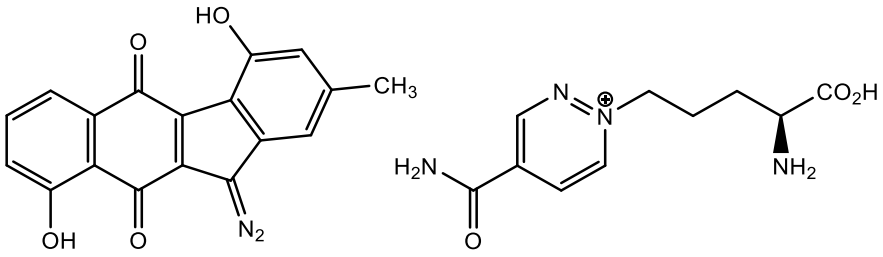
Figure 1: General resonance structures of diazo compounds.

1.1.2 Occurrence of diazo groups

Natural products and their derivatives have great interest in medicinal chemistry, especially for drug screening. They have been recognized for several years as a source of new therapeutic agents and structural diversity. Additionally, they are recognized biological function modifiers. Drug discovery is becoming increasingly challenging, which stems from the need to find new viable and robust candidates,

making naturally occurring compounds an attractive possibility.[8] Most of them are relatively stable and therefore less prone to cause toxic side effects, making them good candidates to further studies. Nevertheless, there are also some reactive compounds in nature that have the ability to, for example, alkylate DNA or generate radical species who are hazardous to living organisms.[9]

N-N bonds are not frequent in natural compounds, but they do exist and, surprisingly, are present in compounds with remarkable structural diversity. These include hydrazines, hydrazones and, among several others, diazo compounds (**Figure 2**).[10] The majority of these compounds are synthesized by different microorganisms and,, although the respective mechanisms still remain unclear,[11] there are reports of various postulated biosynthetic pathways.[2,10–13] For example, *Streptomyces cremeus* was object of studies which concluded that a nitrous acid biosynthetic pathway was responsible for the production of diazo compounds, using nitrous acid as the diazotizing reagent for cremeomycin, a diazo compound with antimicrobial properties.[12]



prekinamicin pyridazomycin

Figure 2: Natural molecules containing diazo groups.

One of the first examples of biomolecules containing diazo groups to be isolated were amino acids. Some of these have inclusively reported antibacterial and anticancer activity mainly due to being analogues of glutamate.[10,14] For example, 6-diazo-5-oxonorleucine (DON) has entered in clinical trials for having showed its beneficial activity against carcinomas, lymphomas, and Hodgkin's disease.[15] An additional example is 5-diazo-4-oxonorvaline (DONV) which is an asparagine analogue and has proven to be very useful in medicine by inhibiting the growth of asparagine-depending tumors due to its ability to interfere with asparagine synthesis and utilization.[16,17] Additionally DONV is a specific L-asparaginase inhibitor, which is a class of compounds used in the treatment of leukemia.[18] These findings are very important as

they also highlight the biocompatibility of certain diazo compounds. Usually these are α-diazocarbonyl compounds because they are significatively more stable in aqueous media. This occurs due to the stabilizing effect of the carbonyl in the α position. Some examples of diazo-containing amino acids are shown in **Figure 3**.[6,10]

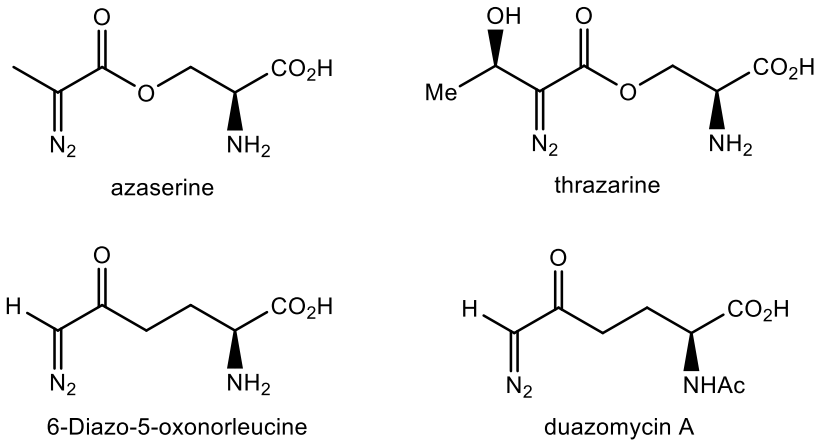


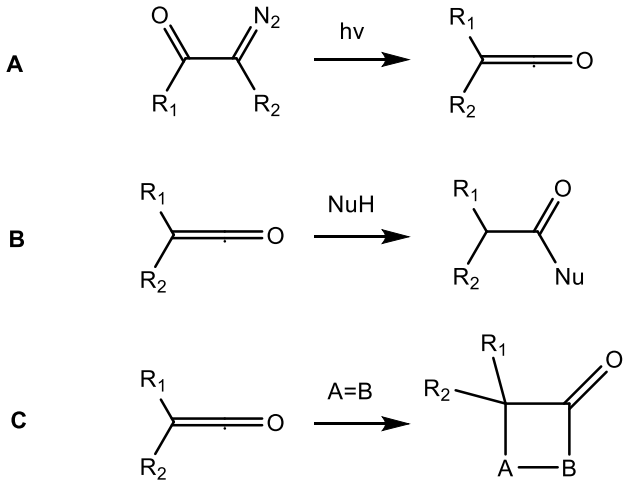
Figure 3: Amino acids containing diazo groups.

1.1.3 Diazo compounds as molecular probes

In recent years, molecular probes have found widespread application as a powerful methodology to elucidate the interaction between proteins and small molecules. A molecular probe is usually designed starting from the structure of a known small molecule ligand for a certain protein, with the insertion of a highly reactive unit in its structure. Incubation of a molecular probe with the protein of interest, followed by probe activation, leads to the formation of a very stable adduct between these two entities that can be further analyzed with various techniques. This analysis can lead to the characterization of the interaction between the probe and the target protein, allowing to gain insight on the binding site, active conformation and several other biological data regarding the interaction of the ligand with the protein. In this framework, it is important that the structure of the probe resembles the one of the small molecule modulator in exam, in order to rationalize the data obtained with this approach. For this reason, the ideal chemical groups for the development of chemical probes are small reactive handles that can be easily incorporated on a known scaffold with limited interference on its tridimensional structure.[19–21] Very few functional groups have been shown to

comply with all the requisites to be useful probes.[22] Examples include ketones, aldehydes, azides and diazos.

Photoaffinity probes have recently been gaining attention in medicinal chemistry regarding drug discovery. They are powerful tools that are used for studying protein-ligand interactions, giving insight on their structure, conformational changes, binding sites and functions.[23] Diazo compounds have been extensively used as photoaffinity probes[24,25]. In fact, upon irradiation by light of the appropriate wavelength, they fragment into molecular nitrogen and a carbene. After this point two reactions may occur, the carbene can either undergo an insertion reaction or suffer a Wolff rearrangement (**Scheme 1**). The desired reaction is the first one, having the carbene interact with a close nucleophile of a target biomolecule. Nevertheless, a carbene is a very reactive species and there is a possibility of undergoing an intramolecular reaction. Photoaffinity probes using diazo compounds are designed to minimize the possibility of undergoing Wolf's rearrangement.[26–28] They have been described in the literature as capable of reporting the presence of cell-surface glycosylation,[22] acetylcholine, nucleotide and steroid receptors,[25] labeling antibody combining sites[29] and examining the structure of biological membranes.[30]



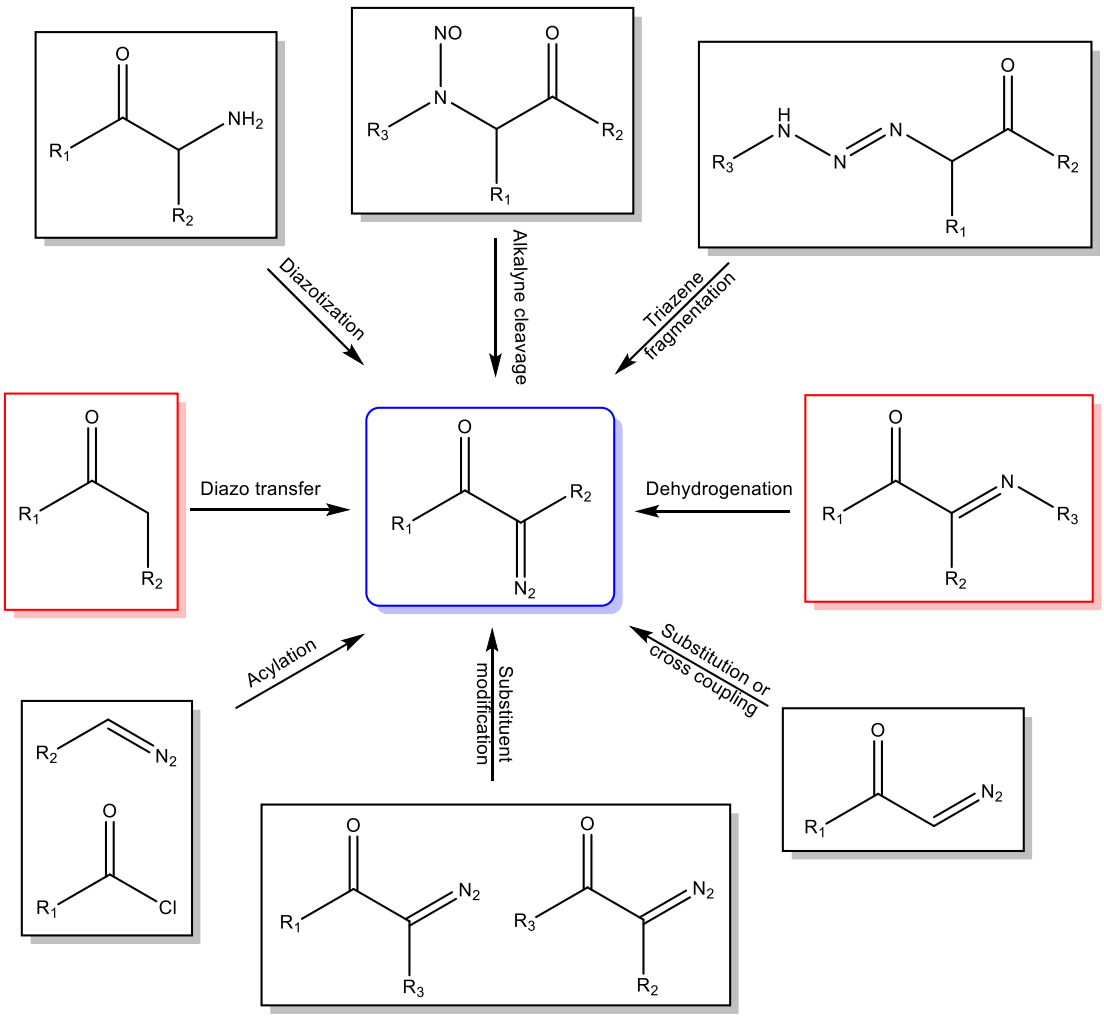
Scheme 1: A-Carbene formation from a diazo compound; B-Insertion reaction from a carbene. C-Wolff rearrangement from a carbene.

1.1.4 Synthetic methodologies

Diazo compounds, as mentioned above, tend to be unstable and hazardous. Meanwhile α-diazocarbonyl compounds are more stable in aqueous media and easily prepared.[31] The synthetic methods here described will focus on this particular group of diazo compounds because the objective of this work is to synthesize a biocompatible molecule.

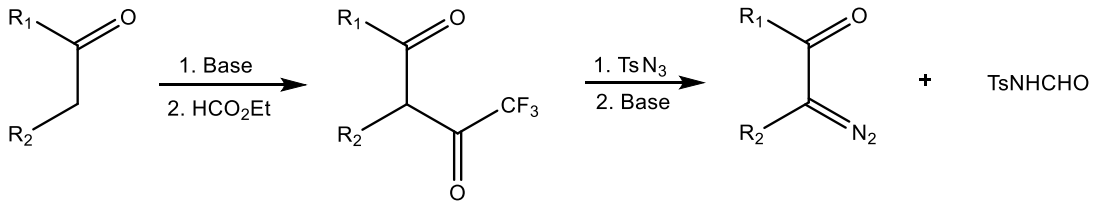
Several methods have been used to achieve the synthesis of diazo compounds. These include diazo transfer reactions, dehydrogenation of hydrazones, tosylhydrazones and oximes, diazotization of primary amines, acylation of diazoalkanes, alkaline cleavage of N-alkyl-N-nitroso compounds, triazene fragmentation, substitution and cross-coupling at the diazomethyl carbon and substituent modification in diazocarbonyl compounds (**Scheme 2**).[32] For the purposes of this work only the first two methods mentioned will be explained in detail.

The diazo transfer methodology involves, as the name indicates, the transfer of a pre-existing diazo group from a donor (for example, sulfonyl azide) to an acceptor. The acceptor must be a carbonyl compound with mild acidity in the α position, poorly acidic substrates require prior activation in order to have a reactive α proton.[32] This strategy was investigated for the first time by Dimroth in 1910,[33] but the general method was only established in 1964.[34] There are several factors that affect this type of reaction, being the most important ones the choice of solvent and base. The reaction is remarkably effective when employing dicarbonyl compounds due to the increased acidity of the α proton of this class of compounds.[35]



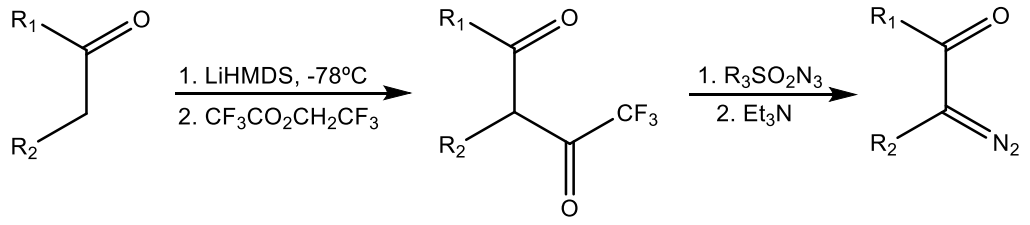
Scheme 2: Methods more widely used for the synthesis of diazocarbonyl compounds.

To improve the efficiency of the aforementioned reaction, a new methodology, "deformylating diazo-group-transfer", was developed. This strategy involves the activation of a substrate via Claisen condensation of a ketone with ethyl formate in the presence of sodium to generate 1,3-dicarbonyl compounds. Afterwards the formyl group is removed in the process of the diazo transfer, occurring fragmentation of the intermediate resulting in the formation of the diazo compound (**Scheme 3**).[36]



Scheme 3: Regitz diazo transfer method.

The development of this technique was important because it allowed the synthesis of cyclic α-diazocarbonyl compounds without the use of diazomethane which, due to its toxicity, is a very hazardous reagent.[32] A few years later, changes were made to this method and the use of a trifluoroacetyl group as an activator was reported, which eliminated some of the existing limitations to synthesize α,β-unsaturated diazoketones (**Scheme 4**).[37–39]

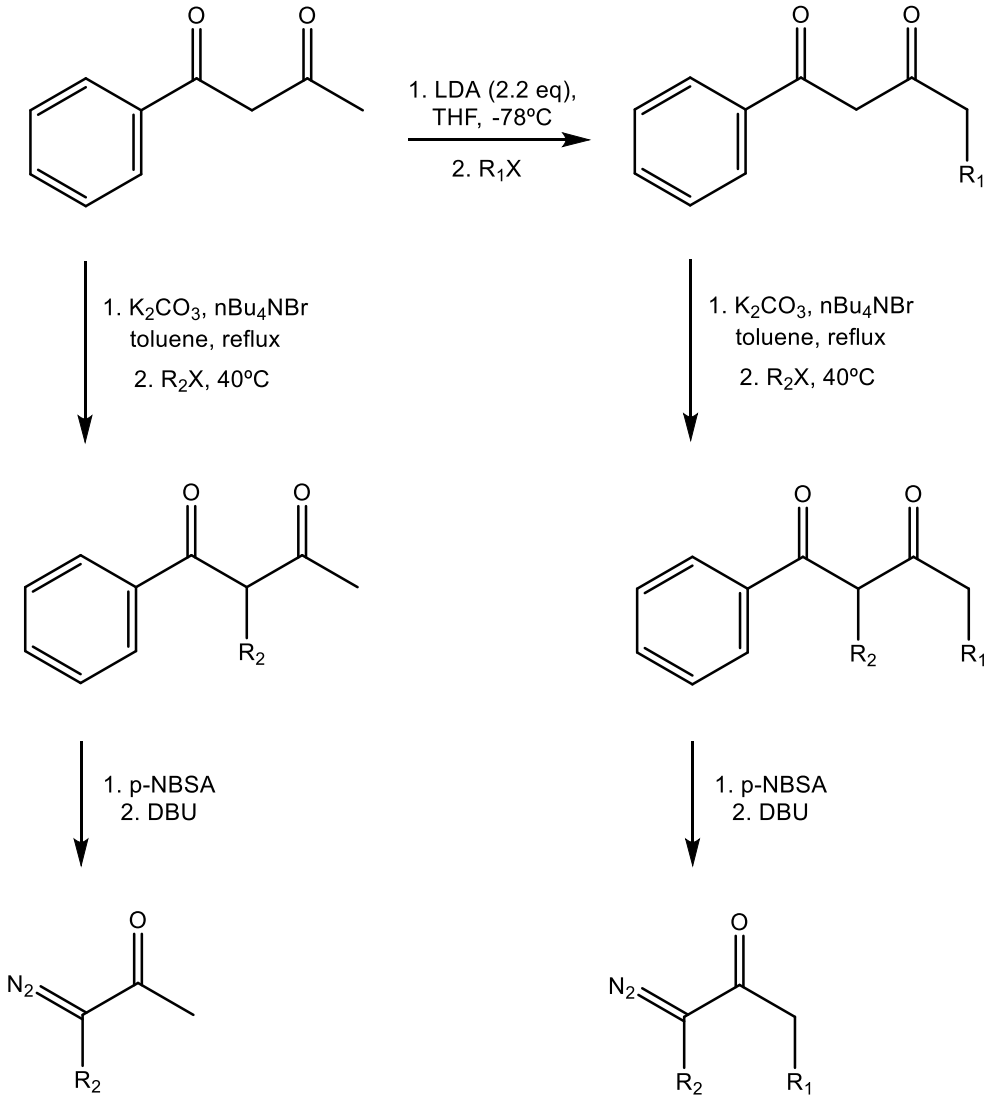


Scheme 4: Danheiser modification of the Regitz's method.

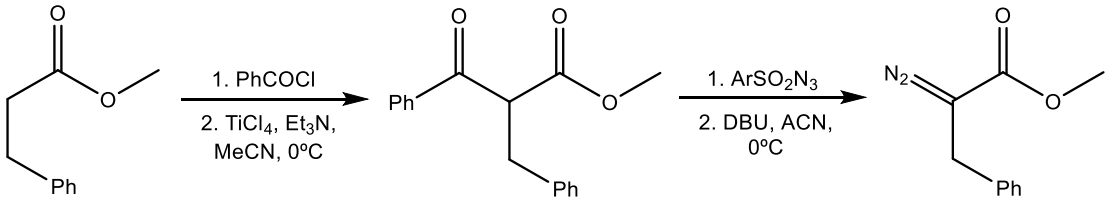
Taber and co-workers also performed a useful modification to the original procedure reported by Regitz and co-workers that allowed the synthesis of unsymmetrical α-diazoketones. This method involves an initial activation of the substrate by benzoylation. The resulting benzoylacetone can be alkylated in the -α position or in both -α and -γ positions. The final step happens in two phases. First occurs the debenzoylation and, in second place, the diazo transfer via *p*-nitrobenzenesulfonyl azide (p-NBSA) and DBU as base (**Scheme 5**).[40] A few years later Taber and co-workers performed some modifications to this methodology substituting the previous method of benzoylation with a titanium chloride-mediated one in order to activate the ester, followed by the diazo transfer which allowed the reaction to occur under mild conditions (**Scheme 6**).[41] A few years later, methods using succinimidyl diazoacetate were developed to perform direct diazoacetylation of amines, phenols, thiophenol and peptides under mild conditions in good yields (**Scheme 7**).[42,43]

The modifications to the original method reported by Regitz and co-workers stem from the necessity to have readily available diazo transfer reagents with thermal stability and that could limit the formation of sulfonamide by-products which can be difficult to remove completely.[32] As mentioned before, diazo transfer reactions require, besides an acceptor, a donor of the $-N_2$ group designated by diazo transfer reagent. These reagents were developed and used throughout the years and include imidazolesulfonyl azide salts, sulfuryl diimidazole,[45] benzotriazole-1-sulfonyl azide,[46] nonafluorobutanesulfonyl azide[47] and 2-azido-1,3-dimethylimidazolinium salts.

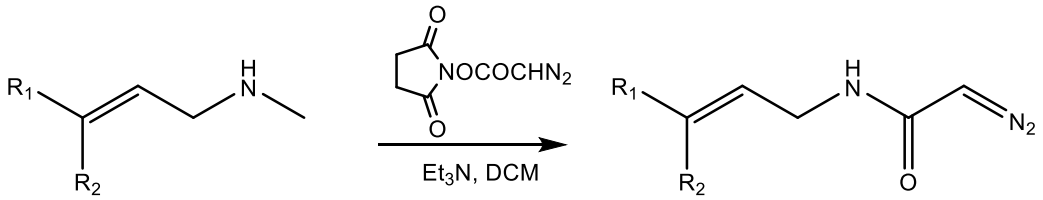
Although the stability of the reagents is of high significance, the changes made throughout the years to the original methodology also have the objective of exploring and developing new and improved strategies to obtain diazocarbonyl compounds. One example of this are diazo transfer reactions in ionic liquids such as 1-butyl-3-methylimidazolium salts.[49]



Scheme 5: Taber diazo-transfer method with pre-benzoylation.



Scheme 6: Taber strategy modification.



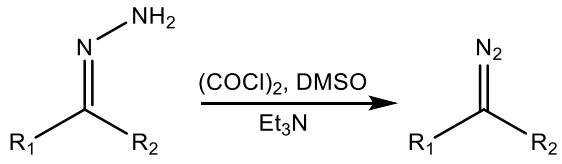
Scheme 7: Diazo transfer method using succinimidyl diazoacetate.

Efforts towards environmental friendly methods to achieve these compounds were also performed with success by using a safer polymer-supported benzenesulfonyl azide and a catalytic amount of base in water.[50] Another interesting advancement made was the use of a magnetic benzenesulfonyl azide as a diazo transfer reagent, allowing for easy separation of the sulfonamide by-product.[51] Lastly, diazo transfer reaction were also explored in the context of a continuous process with the tosyl azide being formed *in situ* and used in sequential diazo transfer reactions with several types of acceptors. This strategy allows the large scale synthesis of these compounds with high purity without having to go through the process of column chromatography and it minimizes the risks of working with diazo compounds.[52]

The dehydrogenation of hydrazones was one of the first methods established for the synthesis of diazo compounds.[53] This type of reaction occurs between metallic catalysts, usually heavy metals, who act as oxidizing agents towards hydrazine (**Scheme 8**). Examples of these include lead (IV), mercury oxide and manganese dioxide.[54]

Scheme 8: General reaction of hydrazone dehydrogenation.

Alternative routes have been developed to avoid the use of heavy metals. One example is the *in situ* formation of chlorosulfodimethyl chloride (Swern reagent) via reaction between dimethyl sulfoxide (DMSO) and oxalyl chloride in the presence of triethylamine (**Scheme 9**).[55]

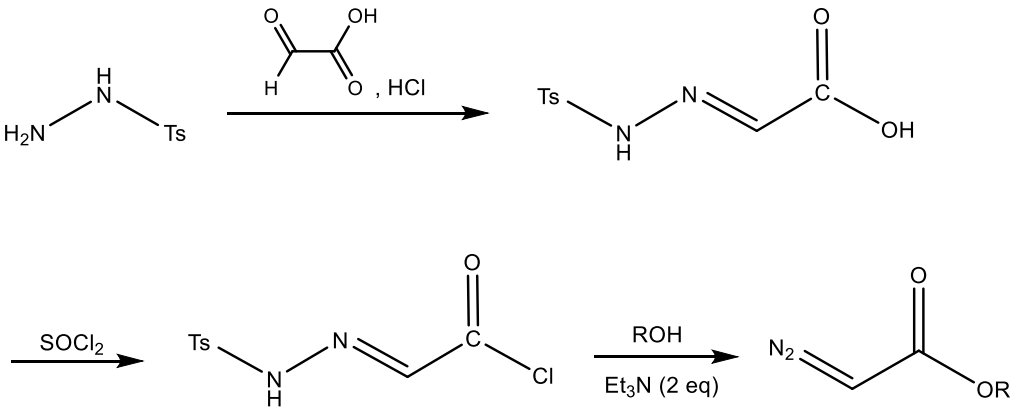


Scheme 9: General method to the synthesis of diazo compounds via Swern's reagent.

Cleavage of toluenesulfonyl hydrazones, or Bamford-Stevens reaction, is a commonly used method to obtain diazo compounds from hydrazones. The tosylhydrazones used are usually synthesized from tosylhydrazides, aldehydes or ketones to provide the correspondent diazocarbonyl compound (**Scheme 10**).[53,56]

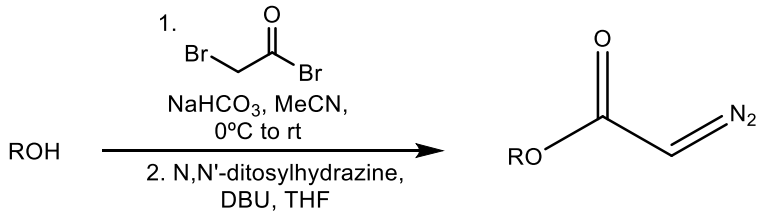
Scheme 10: Bamford-Stevens reaction.

In 1968 House performed a modification of the above method that allowed the synthesis of α-diazoesters which were difficult to obtain previously. This reaction is based on the conversion of glyoxylic acid to its corresponding tosylhydrazones followed by reaction with thionyl chloride. In the last step, two equivalents of triethylamine are used to convert the hydrazone ester to the corresponding diazoester (**Scheme 11**).[57]



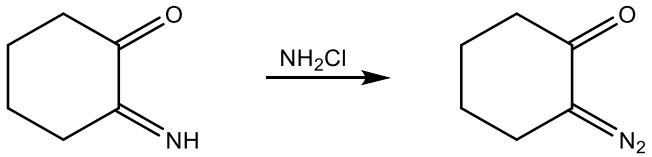
Scheme 11: House modification of the Bamford-Stevens reaction.

Although the House method represents a significative improvement towards the Bamford-Steven reaction in terms of alcohol conversion to diazoacetate, it takes two steps to prepare the reagent and the third step leads to the isolation of the desired product.[57] In 2007 a new method with fewer steps and shortened reaction time to prepare these compounds was reported by Fukuyama and co-workers. This methodology involves treating alcohols with ditosylhydrazine and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) to generate diazocarbonyl compounds in good yields (**Scheme 12**).[58]



Scheme 12: Fukuyama method to obtain diazocarbonyl compounds.

Dehydrogenation of oximes, first reported in 1915 by Forster, is another method towards the synthesis of diazocarbonyl compounds. It consists in the preparation of diazocarbonyl compounds from the reaction of α-ketoximes, mainly cyclic ones, with chloramines (**Scheme 13**).[59]



Scheme 13: Forster reaction.

1.2 Boronic Acids

1.2.1 Structure and Properties

Boronic acids (BAs) are organic compounds characterized by having a trivalent boron atom which possesses two hydroxyl groups and one carbon-based substituent (**Figure 4**). In BAs, boron has six valence electrons leaving a vacant *p* orbital which results in a sp^2 hybridized atom, giving BAs a trigonal planar geometry.[60]

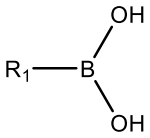
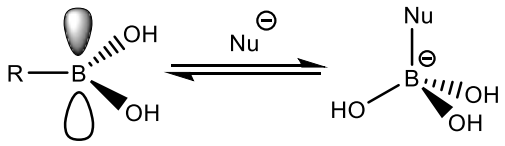


Figure 4: General structure of boronic acids.

The boron element is very interesting because it occupies the same period as carbon in the periodic table but with one less electron. This peculiarity makes it very useful in organic and medicinal chemistry because it can be used as a structural analogue of carbon.[61] The vacant *p* orbital makes boronic acids behave as mild Lewis Acids,[60] having most phenylboronic acids a pKa ranging between 4.5 and 8.8.[62] This leads to an equilibrium, under physiological conditions, where there is an

interconversion between a sp^2 trigonal planar boron and an anionic sp^3 tetrahedral one (**Scheme 14**).[63] This property allows BAs them to readily establish reversible covalent bonds with oxygen and nitrogen nucleophiles.[64] On the other hand, BAs can also behave as Brönsted acids when they play the role of proton-donor receptor, regarding the hydroxyl groups but there is no data that proves the stability and selectivity of these complexes in solution.[65]



Scheme 14: Equilibrium between boron sp^2 and sp^3 structures under physiological conditions.

BAs were first isolated in 1860[66] and are not found in nature. They are derivatives of boric acid and obtained exclusively by chemical synthesis. These compounds are usually solid, considered relatively stable to atmospheric oxidation and have normally a long shelf life. They also have a low toxicity and, due to their ultimate degradation product being boric acid, they are considered environment friendly compounds as this compound is harmless towards humans.[60]

Recently there has been an increased interest in BAs and their status evolved from relatively neglected compounds to a prime class of synthetic intermediates and potential and diverse pharmacological agents,[60,61,64] such as, Bortezomib, as an anti-cancer drug,[67] Tavaborole, an antifungal,[68] Crisaborole, for the treatment of eczema and atopic dermatitis,[69] the β-lactamase inhibitor Vaborbactam[70] and Ixazomib for the oral treatment of multiple myeloma (**Figure 5**).

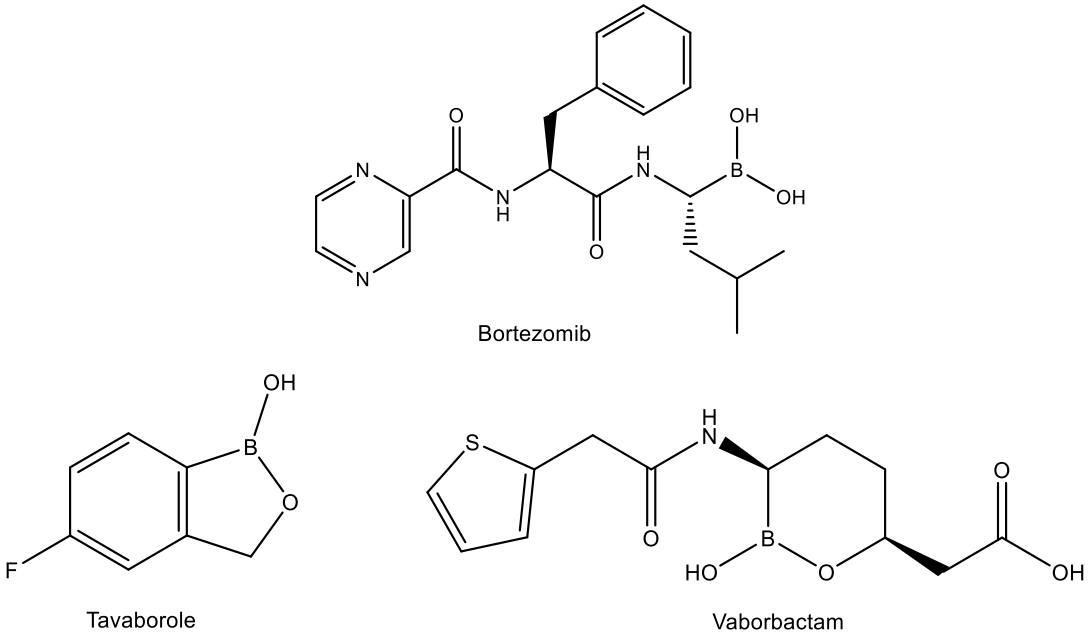


Figure 5: Examples of boronic acid-containing pharmaceutical agents.

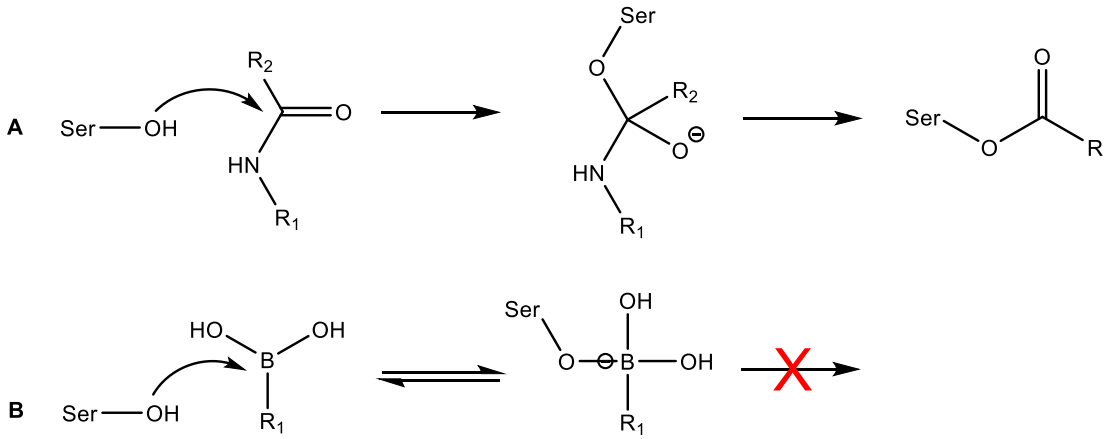
1.2.2 Role of boronic acids in chemistry

These compounds are important intermediates in organic synthesis, materials, bioorganic, medicinal chemistry and chemical biology.[61] In the field of organic chemistry they are mainly used for Suzuki cross-coupling reactions,[71] aromatic functionalization,[72] Diels-Alder reactions,[73] protection of diols,[74] selective reduction of aldehydes,[75] carboxylic acid activation,[76] synthesis of asymmetric molecules[77] and as template in organic synthesis.[78] In materials science, BAs are important in areas such as construction of polymers with reversible properties,[79] separation and purification of glycosylated products,[80] and stimuli-controlled drug delivery.[81] In bioorganic chemistry BAs are commonly used as recognition moiety for the design and synthesis of sensors for carbohydrates,[82,83] reactive oxygen species, catecholamines[83] and aminoacids.[84] In medicinal chemistry they are important mainly as enzymes inhibitors[61,85] and boronic neutron capture therapy (BNCT).[86] Lastly, in the field of chemical biology they are used for the recognition and sensing of tetraserine motifs in proteins,[87] development of new Magnetic Resonance Imaging (MRI) contrast agents[88] and BA-modified proteins for various sensing and purification applications.[89]

Most of the aforementioned applications of BAs, especially the biological ones, stem from their unique reactivity with nucleophiles in water. In fact, when they react

with nucleophiles, of which diols are the most explored, they establish an equilibrium in water between a trigonal planar structure and a tetrahedral one. Several biomolecules contain diol groups, such as catecholamines, glycoproteins, saccharides, nucleosides and nucleotides.[90] The BAs characteristics mentioned make them interesting molecules to study. They have applications in linker engineering, payload attachment and bioconjugation.[64] Various boron-containing molecules have been developed over the course of the years, mostly protein inhibitors.[61,91] BAs are indeed a promising class of enzyme inhibitors, mainly of serine proteases.[92,93]

Because of the equilibrium between trigonal planar and tetrahedral structures that BAs establish under physiological conditions, they are well suited to act as flexible anchoring elements, having the potential to stabilize or destabilize protein targets.[94] Serine proteases are one of the largest classes of studied proteases.[95] These proteins are object of great interest due to their well-characterized role in many physiological and pathological processes caused by deficiencies in the regulation of the activity of proteolytic enzymes.[96] The substrate of these proteins binds in the active site forming a complex that exposes the peptidic bond to nucleophilic attack by the hydroxyl side chain of the serine residue present in the site.[97] The referred mechanism and the equilibrium established by BAs under physiological conditions makes them a prime class of hydrolytic enzyme inhibitors (**Scheme 15**). In fact, the tetrahedral adduct formed with the protein has a very close relationship with the true intermediate, making it suitable for this application.[98–100]



Scheme 15: A-General mechanism of action of proteolytic enzymes in the active site. B-General mechanism of enzyme inhibition mediated by boronic acids.

Another important application of BAs is as stimuli-responsive sensors. The world is made up of elements, ions and molecules that continuously interact with each other in several different ways. Scientists are always in the search for new and improved tools that can help them understand these processes, and for that it is necessary to establish what happens to a molecular level. To achieve this goal, a lot of time is spent in the development of tools such as sensors that can recognize specific molecules. There are two main features an ideal sensor must comply with. The first one is to have a specific recognition site that interacts tightly with entities such as ions, carbohydrates and peptides. This interaction can be covalent, non-covalent or reaction-based. The second requisite is that the changes resulting by these interactions can be monitored effectively and precisely.[83,101]

Recently, a series of sensors, including fluorescent ones, using BAs have been developed to take advantage of the dynamic covalent bond formation due to its peculiar equilibrium in aqueous media.[83] Hydrogels containing BAs combine the properties of the latter and the characteristics of said dynamic material, conferring it new functions such as sugar responsiveness, reversibility and self-healing. Hydrogels are three dimensionally crosslinked hydrophilic polymer networks that expand in aqueous solutions but do not dissolve, retaining their shape.[102] The key property of this type of material is the fact that the expansion and/or contraction degree can be altered by external stimuli such as glucose concentration.[103,104] Certain functional groups provide the hydrogel with unique physical properties in terms of expansion/contraction and three-dimensional structure. Moreover these materials are usually biocompatible, giving them widespread applicability.[104]

Carbohydrate sensors are one of the most interesting and established application for BA derivatives due to the previously referred equilibrium in water.[83] These biomolecules are widely distributed in the organism and have a key contribution to the maintenance of its normal functions, including production of energy for basic processes, regulation of the nervous system and as structural blocks.[105] In consequence, the recognition of carbohydrates is of great interest in chemical biology and in life sciences. For instances, glucose plays an important role in several biological processes and, for that, is a basic necessity of living organisms. Abnormal glucose levels in individuals are usually a signal that alerts for the possibility of an underlying medical

condition. The development of selective glucose sensors has since become of great importance for clinical and biomedical applications and a crucial goal in the field of BA chemistry. The selectivity for different carbohydrates can be obtained via specific molecular design and this type of technology is in constant evolution, allowing the increase in the sensibility and specificity of the sensors.[83] Moreover, the wide range of BA-derived carbohydrate sensors does not only include glucose but also ribose,[106] sialic acids,[107] glucosamine,[108] ATP[109] and amyloid β-plaques (**Figure 6**).[110]

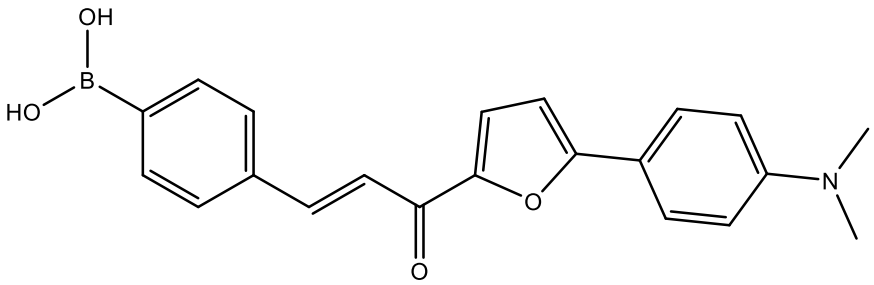


Figure 6: Boronic acid sensor for amyloid β-plaques.

BAs have also found widespread application in the field of bioconjugation.[64] Bioconjugates are multifunctional constructs where biological molecules such as peptides, proteins and nucleic acids are modified with specific payloads in order to give them useful and new properties.[64,111,112 113]

BAs have also been explored as prodrugs and self-immolative modules. Phenylboronic acids are known to convert rapidly to their corresponding phenols by reacting with hydrogen peroxide.[64] This property is very interesting and useful in linker design for active compounds that target cancer cells. This derives from the altered metabolism of the referred cells. The alterations include increased rates of glycolysis alongside with slightly reduced mitochondrial respiration. In normal mammalian cells, the mitochondria are the major cellular organelles responsible for respiration. The electron transport chains in the inner membrane of this organelle are believed to be responsible for most of the oxygen consumption and the primary source of reactive oxygen species (ROS) during metabolism. Studies have shown that some types of cancer cells have an increased steady-state oxygen level which proportionally leads to an increase in the amount of ROS formed. This may provide a biochemical target more selective towards human cancer cells, enhancing cytotoxicity of pharmaceutical agents targeting those types of cancer.[114] Exploration of this type of behavior led to ROS-

triggered prodrugs by replacing the phenol moiety in the original drug for a BA one. In fact, it was described a BA-prodrug of Irinotecan sensible to low concentrations of ROS that shows better *in vivo* activity in glioblastoma models than the original one.[115] High concentration of ROS are also present in inflamed tissues and inflammatory prodrugs have also been studied by borylating an existing drug, leading to a better circulation efficacy and half-life than the original one.[116]

2 Rationale and Goals

Affinity probes have become relevant tools to better understand the mechanisms that underly the function of their molecular targets. One of the most important requisites for a probe is to bind tightly to a specific target and for that interaction to be measured in a precise way. BAs are known to form reversible complexes with hydroxylated compounds under physiological conditions, shifting between a trigonal planar sp^2 structure to a tetrahedral sp^3 one. In fact, this type of compounds can be used as a recognition moiety for an affinity probe that targets -OH rich molecules such as proteins and carbohydrates. Diazo compounds, on the other hand, are easily decomposed by light, of the appropriate wavelength, in their respective carbene, binding tightly to their target.

In this work, the objective was to synthesize a novel α-diazocarbonyl molecule containing a BA moiety: 2-diazo-N-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide, **compound 5 (figure 6)**.

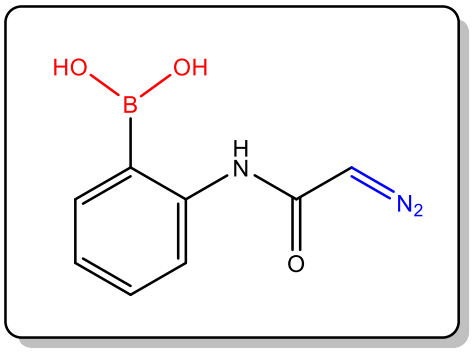


Figure 7: Molecular structure of the desired compound.

Once synthesized, our objective is to incubate the molecule, under physiological conditions, with serine-rich proteins, which can form covalent interactions with the BA moiety of our molecule thanks to their hydroxylated side chains. After the complexation of our molecule with the protein, we envision to activate the diazo group with an appropriate stimulus (light, rhodium catalyst) in order to trigger carbene formation and subsequent covalent attachment to the protein. If the recognition by the BA moiety is proven to occur, there are several attractive applications for this compound. Sialic acid, for example, is a carbohydrate containing several hydroxyl groups that is present at the cellular surface. In cancer cells it has a significantly higher expression when compared

to healthy ones, making it a promising molecular target.[107] Other interesting targets are serine-rich repeat proteins (SRRPs). These domains are expressed at the surface of many Gram-positive pathogens and have been demonstrated to have an important role in the adhesion of the pathogens to the tissues and in the development of invasive disease.[117] Tools to recognize and label this type of proteins are of great value to clinical and analytical practice.

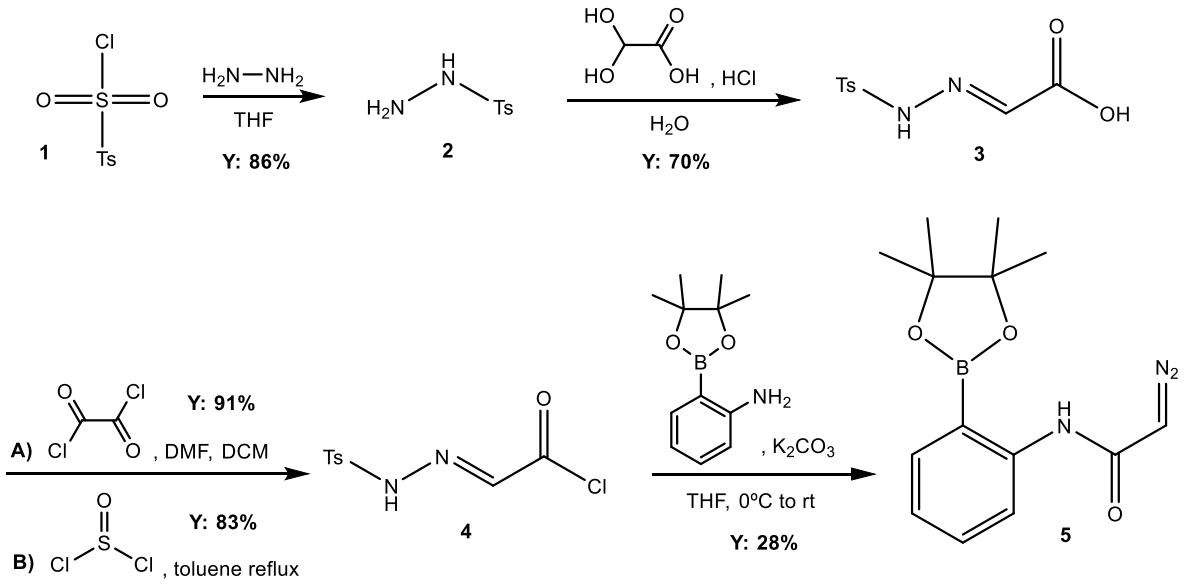
3 Materials and Methods

3.1 General Remarks

The reagents and solvents used were acquired from Sigma-Aldrich, Merck, Alfa Aesar, Fluorochem or TCI, unless otherwise noted and were used without further purification. The solvents used in the reactions weren't target of further purification and in case of air or moisture sensitive reactions they were obtained in anhydrous conditions by distillation under nitrogen. Air and moisture sensitive liquids were transferred to the reaction mixture recurring to a syringe coupled to a stainless-steel cannula. Evolution of reactions was followed by TLC using silica gel 60 F254 Aluminum plates and revealed by UV light at 254 nm and 325 nm or stain solution of potassium permanganate and/or ninhydrin with posterior heating. Flash chromatography and preparative flash chromatography were performed using Silica gel 60 from Aldrich. The obtained compounds were characterized by NMR in a Ultrashield Bruker Fourier 300 spectrometer. The IR spectra were obtained using a Bruker Alpha II FT-IR.

3.2 Methods

3.2.1 Synthetic Pathway A



Scheme 16: Synthetic scheme of pathway A.

4-methylbenzenesulfonohydrazide (2): Hydrazine monohydrate (20.6 mmol, 1.032 g, 1 mL, 5 eq) was slowly diluted in distilled water (0.331 mL) and the solution put into a funnel for dropwise addition. In parallel, *p*-toluenesulfonyl chloride (4.12 mmol, 0.785 g, 1 eq) was dissolved in THF (2.108 mL) and kept in water iced bath at 10°C<T<20°C. The first solution was then added dropwise to the second one under controlled temperature (T<10°C). After the addition was complete, the reaction was left stirring for 15 minutes and then was put in a separatory funnel. The organic phase was separated from the aqueous one, washed with brine, dried with magnesium sulfate, collected and filtered through celite. The resulting filtrate was put under strong magnetic stirring and two volumes of distilled water were added to it. The obtained solution was put in the refrigerator overnight. The resulting precipitate was filtered through a Buckner funnel and washed several times with cold distilled water and then air dried. ^{1}H-NMR spectrum matches the one described in literature.[118] Yield: 86%.

(E)-2-(2-tosylhidrazone)acetic acid (3): 2,2-dihydroxyacetic acid (2.7 mmol, 0.249 g, 1 eq) was dissolved in water (2.488 mL) at 65ºC and kept under magnetic stirring. To this solution, a suspension of 4-methylbenzenesulfonohidrazyde (2.7 mmol, 0.500 g, 1 eq) in HCl 2.5 M aqueous solution (3.8 mmol, 0.115 mL, 1.4 eq), previously heated at 65°C, was added causing almost instant precipitation. The resulting suspension was left under magnetic stirring at 65°C for 15 minutes and afterwards was left to cool down at room temperature and then put in the freezer overnight. A solid was obtained and filtered through a Buckner funnel and air dried for two days. Then it was put in a flask and kept in the vacuum pump overnight. The resulting white solid was recrystallized by putting it in a flask equipped with a condenser on top and by first dissolving it in a minimal amount of boiling ethyl acetate. Then hexane was added dropwise until the solution became cloudy, after which ethyl acetate was added again dropwise until the solution became clear again. Afterwards the solution was left in the freezer overnight. The next day the solution was filtered over a Buckner funnel and the resulting solid washed with a cold mixture of Hexane and Ethyl acetate (2:1). The white solid obtained was dried at the vacuum pump overnight. ^{1}H-NMR spectrum matches the one described in literature.[119] Yield: 70%.

(E)-2-(2-tosylhidrazono)acetyl chloride (4): This compound was synthesized with two different methods.

Method 1:

In a flame-dried flask under argon atmosphere, (E)-2-(2-tosylhydrazono)acetic acid (5.49 mmol, 1.33 g, 1 eq) was suspended in freshly distilled toluene (6.46 mL). Thionyl chloride (10.98 mmol, 0.801 ml, 2 eq) was then slowly added dropwise to the flask. The resulting mixture was then heated to reflux under argon atmosphere. The reaction was stopped after 2 hours by quickly cooling the flask to room temperature with a water bath. The resulting solution was then filtered through celite and the filtrate concentrated under reduced pressure, yielding a solid. The crude was dissolved in warm dry toluene, then hexane was added until the formation of precipitate was observed. This solid was filtered over a Buchner funnel and washed with hexane. No further purification was performed. The product obtained was pale yellow crystals, with ^{1}H-NMR corresponding to the one reported in the literature.[119] Yield: 83%

Method 2:

In a flame-dried flask under argon atmosphere, (E)-2-(2-hidrozono)acetic acid (5.4 mmol, 1.300 g, 1 eq) was suspended in freshly distilled DCM (26 mL). To this mixture DMF (0.11 mmol, 8.4 μL, 0.02 eq) and oxalyl chloride (5.4 mmol, 470 μL, 1 eq) were added. The resulting mixture was left under magnetic stirring overnight. Afterwards it was filtered through celite and the solvent evaporated under reduced pressure. The obtained solid was then redissolved in a minimal amount of DCM and hexane was added dropwise until the solution became cloudy. The resulting mixture was filtered under vacuum through paper filter. No further purification was performed. The product obtained was pale yellow crystals, with ^{1}H-NMR corresponding to what indicated in the literature. Yield: 91%.

2-diazo-N-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide (5): Five different methods were tested for the synthesis of this compound.

Method 1:

In a flame-dried flask under argon atmosphere, (E)-2-(2-tosyl-hydrazono)acetyl chloride (0.58 mmol, 0.150 g, 1 eq) was dissolved in freshly distilled DCM (1.5 mL).

The resulting solution was then added dropwise over a 1-hour period to a stirring suspension of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.58 mmol, 0.127 g, 1 eq) and diisopropylethylamine (DIPEA) (1.74 mmol, 303 μL, 3 eq) in freshly distilled DCM (1.15 mL) and left stirring at 0°C under argon atmosphere. After this was complete, the reaction was kept under stirring for 1 hour and then 3 hours at room temperature. Then it was dissolved in a minimal amount of DCM and precipitated from hexane. Purification was made by flash chromatography using a mixture of ethyl acetate and hexane (6:4). ^{1}H-NMR and ^{13}C-NMR spectra didn't show formation of product.

Method 2:

In a flame-dried flask under argon atmosphere, (E)-2-(2-tosyl-hydrazono)acetyl chloride (0.384 mmol, 0.100 g, 1.24 eq) was dissolved in 190 μL of freshly distilled THF. The resulting solution was then added dropwise, over a 1-hour period, to a stirring suspension of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.310 mmol, 0.067 g, 1 eq), 4-Dimethylaminopyridine (DMAP) (0.077 mmol, 0.009 g, 0.25 eq) and pyridine (0.619 mmol, 50 μL, 2 eq) in 383 μL of freshly distilled DCM and left stirring at 0°C under argon atmosphere. After this was complete, the reaction was kept under stirring 1 hour and then 3 hours at room temperature. Afterwards it was put in a tube and centrifuged. The resulting surnatant was filtered through celite and the filtrate concentrated under reduced pressure. Then it was dissolved in a minimal amount of DCM and precipitated from hexane. Purification was made by flash chromatography using a mixture of ethyl acetate and hexane (6:4). ^{1}H-NMR and ^{13}C-NMR spectra of the isolated fractions didn't show formation of product.

Method 3:

In a flame-dried flask under argon atmosphere, (E)-2-(2-tosyl-hydrazono)acetyl chloride (0.58 mmol, 0.150 g, 1 eq) was dissolved in freshly distilled DCM (1.5 mL). The resulting solution was then added dropwise, over a 1-hour period, to a stirring suspension of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.58 mmol, 0.127 g, 1 eq) and sodium carbonate (1,16 mmol, 0,070 g, 2 eq) in 1,15 mL of freshly distilled DCM and left stirring at 0°C under argon atmosphere. After this was complete,

the reaction was kept under stirring for 1 hour and then 3 hours at room temperature. Afterwards it was put in a tube and centrifuged. The resulting mixture was filtered through celite and the filtrate concentrated under reduced pressure. Subsequently, it was dissolved in a minimal amount of DCM and precipitated from hexane. Purification was made on half the crude by column chromatography and the other half by extraction. The first was made using ethyl acetate and hexane (6:4). The latter was accomplished by first concentrate the crude under reduced pressure and redissolving it in DCM. Then it was extracted 3 times with Hydrochloric acid (0.1 M) and after with sodium carbonate saturated solution 3 times. Magnesium sulfate was used as drying agent. Two fractions were collected: one extracted only with the acid and the other with both acid and base, ending up with the boronic acid on the first and the diazo on the second. They were both concentrated under reduced pressure, put under vacuum overnight and analyzed by ^{1}H-NMR, which showed traces of formed product.

Method 4:

In a flame-dried flask under argon atmosphere, (E)-2-(2-tosyl-hydrazono)acetyl chloride (1.2 mmol, 0.300 g, 1 eq) was dissolved in freshly distilled DCM (3.5 mL). The resulting solution was then added dropwise, using a syringe pump to have it last 1 hour, to a stirred suspension of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1.2 mmol, 0.263 g, 1 eq) and sodium carbonate (12 mmol, 0.720 g, 10 eq) in freshly distilled DCM (2 mL) and left stirring at 0°C under argon atmosphere. After this was complete, the reaction was kept under stirring for 1 hour and then 3 hours at room temperature. Afterwards it was put in a tube and centrifuged. The resulting mixture was filtered through celite and the filtrate concentrated under reduced pressure. Then it was dissolved in a minimal amount of DCM and precipitated from hexane. Purification was made by flash chromatography using a mixture of ethyl acetate and hexane (6:4). ^{1}H-NMR and ^{13}C-NMR spectra were compatible with the expected product. Yield: 9 %.

^{1}H-NMR (CDCl3, 300 MHz) δ: 7.72-7.69 (dd, 2H, J=9Hz), 7.31-7.29 (d, 1H, J=6Hz), 7.10-7.05 (t,1H, J=9Hz), 4.79 (s, 1H), 1.36 (s, 12H).

^{13}C-NMR (75 MHz, CDCl3) δ: 163.84, 142.48, 134.83, 130.78, 123.76, 117.41, 82.97, 48.19, 25.40. The signal of the carbon linked to the boron is missing because of the quadrupolar relaxation of the boron nucleus.

FTIR: diazo band at 2200 cm^{-1}

Method 5:

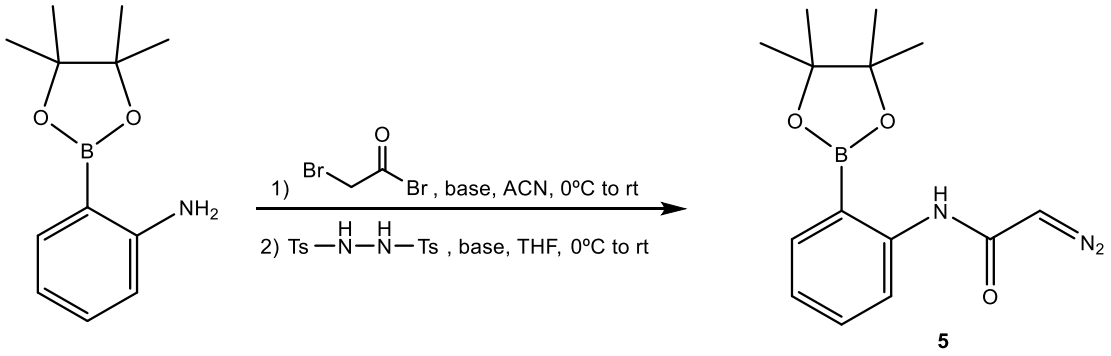
In a flame-dried flask under argon atmosphere, (E)-2-(2-tosyl-hydrazono)acetyl chloride (1.15 mmol, 0.300 g, 1 eq) was dissolved in freshly distilled DCM (4 mL). The resulting solution was added dropwise, over a 1-hour period, to a stirring suspension of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1.15 mmol, 0.252 g, 1 eq) and potassium carbonate (11.5 mmol, 1.591 g, 10 eq) in freshly distilled DCM (3 mL) at 0ºC and left stirring at that temperature under argon atmosphere. After this was complete, the reaction was kept under stirring 1 hour at 0ºC and 3 hours at room temperature. Afterwards it was filtered through a Buckner funnel to remove the remaining potassium carbonate. The resulting mixture was then filtered through celite and the filtrate concentrated under reduced pressure. Purification was made by flash chromatography with a mixture of ethyl acetate and hexane (6:4). ^{1}H-NMR and ^{13}C-NMR spectra showed the formation of the desired product. Yield: 28%

^{1}H-NMR (CDCl3, 300 MHz) δ: 7.72-7.69 (dd, 2H, J=9Hz), 7.31-7.29 (d, 1H, J=6Hz), 7.10-7.05 (t,1H, J=9Hz), 4.79 (s, 1H), 1.36 (s, 12H).

^{13}C-NMR (75 MHz, CDCl3) δ: 163.84, 142.48, 134.83, 130.78, 123.76, 117.41, 82.97, 48.19, 25.40. The signal of the carbon linked to the boron is missing because of the quadrupolar relaxation of the boron nucleus.

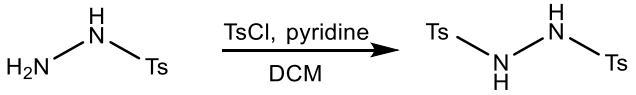
FTIR: diazo band at 2200 cm^{-1}

3.2.2 Synthetic Pathway B



Scheme 17: Synthetic scheme of pathway B.

To perform this synthetic route it was necessary to pre-synthesize the reagent used in step 2, N,N'-ditosylhydrazine, since it was not commercially available.

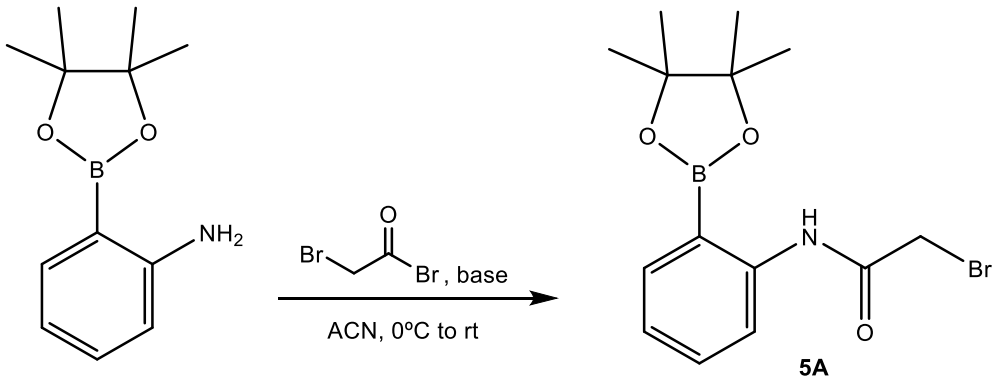


Scheme 18: Synthesis of N-N'-ditosylhydrazine.

N-N'-ditosylhydrazine: In a flame-dried flask under argon atmosphere, freshly distilled DCM (11 mL), *p*-toluenesulfonylhydrazine (10.7 mmol, 2 g, 1 eq) and *p*-toluenesulfonylchloride (16.05 mmol, 3.060 g, 1.5 eq) were added. The resulting suspension was stirred at room temperature while pyridine (16.05 mmol, 1.3 mL, 1.5 eq) was slowly added dropwise over a minute. The resulting mixture turned yellow and homogenous and was left stirring for 1.5 hours. After stopping the reaction, diethyl ether (45 mL) and distilled water (22.5 mL) were added at 0ºC and the resulting mixture left stirring for 15 minutes. The white solid formed was collected on a Buckner funnel via suction filtration and the crystals washed with diethyl ether (22.5 mL). The obtained solid was dissolved in boiling methanol (90 mL) and concentrated under reduced pressure until about half of the methanol evaporated. The resulting suspension was cooled till 0ºC and left in the refrigerator overnight. The resulting precipitate was collected on a Buckner funnel via suction filtration and washed with cold methanol (4.5 mL) and diethyl ether (22.5 mL). The product obtained was white crystals with ^{1}H-NMR spectrum corresponding to what indicated in the literature.[58] Yield: 61%.

2-diazo-N-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide (5): Various conditions were tried to carry out this synthetic route for both step 1 and step 2 as it follows.

Step 1:



Scheme 19: Step 1 of synthetic pathway B.

Method 1:

2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1.45 mmol, 0.300 g, 1 eq) and sodium bicarbonate (4.35 mmol, 0.365 g, 3 eq) were added to a flame-dried flask containing freshly distilled acetonitrile (ACN) (7 mL) under argon atmosphere. Bromoacetyl bromide was then added slowly dropwise at 0ºC. The resulting mixture was stirred at room temperature for 10 minutes and then quenched with distilled water (3 mL). The reaction mixture was extracted with DCM (3 times, 10 mL). The organic phase was then washed with brine and dried with magnesium sulfate. The solvent was evaporated under reduced pressure and the residue put under vacuum for 10 minutes. The resulting crude was analyzed by ^{1}H-NMR, which showed traces of the expected product.[58]

Method 2:

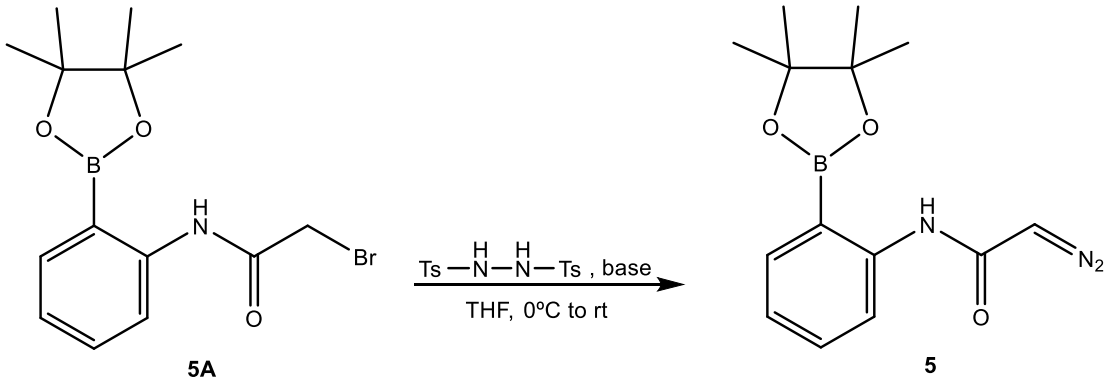
2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1.22 mmol, 0.250 g, 1 eq) and sodium carbonate (12.2 mmol, 1.293 g, 10 eq) were added to a flame-dried flask containing freshly distilled acetonitrile (ACN) (10 mL) under argon atmosphere. Bromoacetyl bromide was then added slowly dropwise at 0ºC. The resulting mixture

was stirred at room temperature for 2.5 hours and then was filtered through a Buckner funnel to remove the remaining carbonate and quenched with distilled water (10 mL). The reaction mixture was then and extracted with DCM (3 times, 10 mL). The organic phase was then washed with brine and dried with magnesium sulfate. The solvent was evaporated under reduced pressure and the residue put under vacuum for 10 minutes. The resulting crude was analyzed by ^{1}H-NMR which showed formation of the expected product. Yield: 77%.

Method 3:

2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1.22 mmol, 0.250 g, 1 eq) and potassium carbonate (12.2 mmol, 1.686 g, 10 eq) were added to a flame-dried flask containing freshly distilled acetonitrile (ACN) (10 mL) under argon atmosphere. Bromoacetyl bromide was then added slowly dropwise at 0ºC. The resulting mixture was stirred at room temperature for 2 hours and then was filtered through a Buckner funnel to remove the remaining carbonate and quenched with distilled water (10 mL). The reaction mixture was then and extracted with DCM (3 times, 10 mL). The organic phase was then washed with brine and dried with magnesium sulfate. The solvent was evaporated under reduced pressure and the residue put under vacuum for 10 minutes. The resulting crude was analyzed by ^{1}H-NMR which showed formation of the expected product. Yield: 53%.

Step 2:



Scheme 20: Step 2 of synthetic pathway B.

Method 1:

Compound 5A was dissolved in freshly distilled THF (7 mL) under argon atmosphere and N-N'-ditosylhydrazine (2.9 mmol, 0.987 g, 2 eq) was added. The resulting solution was cooled to 0ºC and DBU was added slowly dropwise (7.25 mmol, 1.08 mL, 5 eq) and the reaction stirred at room temperature for 10 minutes. The reaction was then quenched with saturated solution of sodium bicarbonate (3 mL). The resulting mixture was extracted with diethyl ether (3 times, 10 mL) and the organic phase washed with brine and dried with magnesium sulfate. The solution was then evaporated under reduced pressure. The crude was purified by flash chromatography with a mixture of hexane and ethyl acetate (6:4). ^{1}H-NMR spectrum did not show formation of the product.[58]

Method 2:

Compound 5A was dissolved in freshly distilled THF (7 mL) under argon atmosphere and N-N'-ditosylhydrazine (3.16 mmol, 0.987 g, 2 eq) was added. The resulting solution was cooled to 0ºC and DBU was added slowly dropwise (7.9 mmol, 1.18 mL, 5 eq) and the reaction stirred at room temperature overnight. The reaction was then quenched with saturated solution of sodium bicarbonate (3 mL). The resulting mixture was extracted with diethyl ether (3 times, 10 mL) and the organic phase washed with brine and dried with magnesium sulfate. The solution was then evaporated under reduced pressure. The crude was purified by flash chromatography with a mixture of hexane and ethyl acetate (6:4). ^{1}H-NMR spectrum did not show formation of the product.

Method 3:

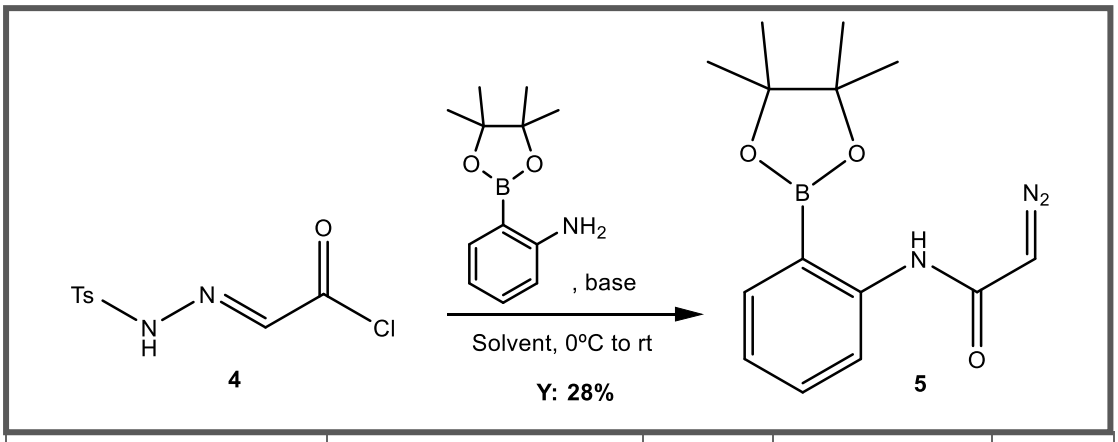
Compound 5A was dissolved in freshly distilled THF (7 mL) under argon atmosphere and N-N'-ditosylhydrazine (3.16 mmol, 0.987 g, 2 eq) was added. The resulting solution was cooled to 0ºC and 1,1,3,3-Tetramethylguanidine (TMG) was added slowly dropwise (7.9 mmol, 1.18 mL, 5 eq) and the reaction stirred at room temperature for 2h. The reaction was then quenched with saturated solution of sodium bicarbonate (3

mL). The resulting mixture was extracted with diethyl ether (3 times, 10 mL) and the organic phase washed with brine and dried with magnesium sulfate. The solution was then evaporated under reduced pressure. The crude was purified by preparative flash chromatography with a mixture of hexane and ethyl acetate (6:4).[120] ^{1}H-NMR spectrum did not show formation of the product.

4 Results

Regarding pathway A, the last step required optimization. Two solvents and several different bases were tested, organic and inorganic ones (**Table 1**).

Table 1: Effect of different bases and solvents on the last step of pathway A.

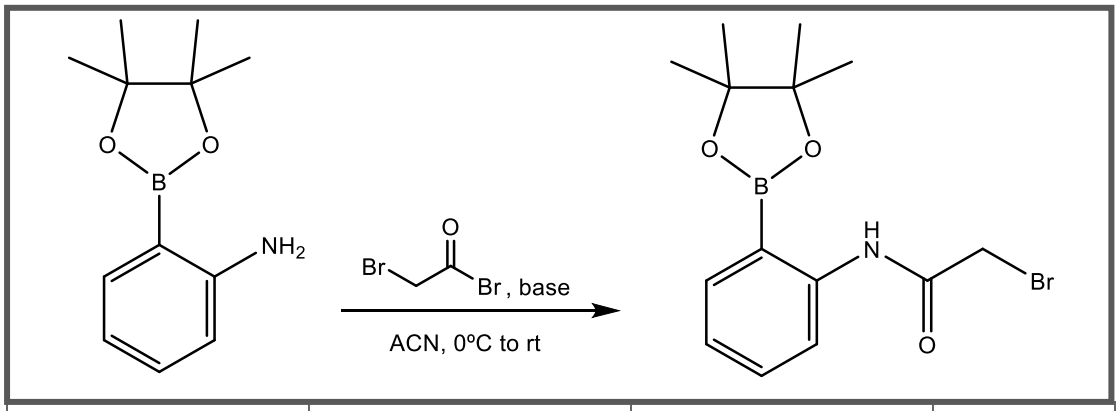


Base	Equivalents of Base	Solvent	Reaction time	Yield
DIPEA	3	DCM	5 hours	N/A
Pyridine/DMAP	2/0.25	THF	5 hours	N/A
Na_2CO_3	2	DCM	5 hours	Traces
Na_2CO_3	10	DCM	5 hours	9%
K_2CO_3	10	DCM	5 hours	28%

N/A: Not applicable.

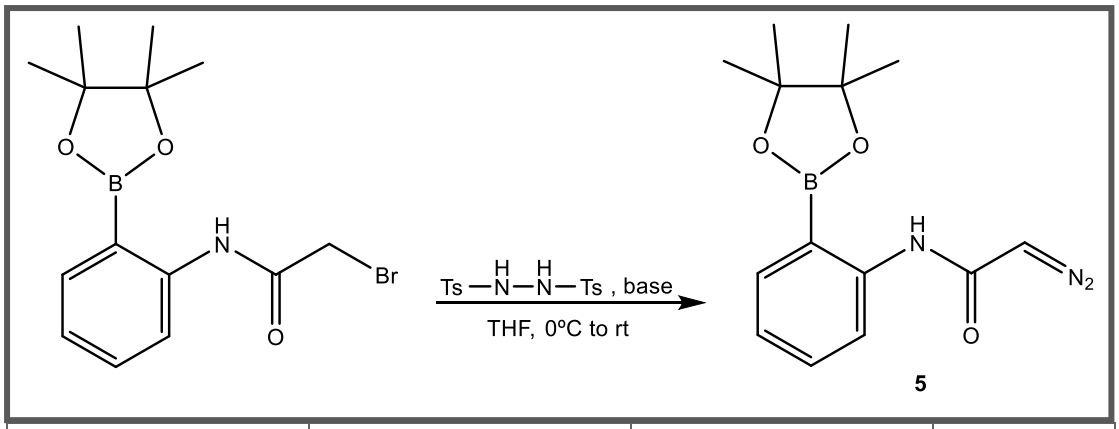
As for pathway B two sets of data were acquired, regarding step 1 and 2 respectively. Three different inorganic bases were tested on step 1 and two different organic bases were tested on step 2. Varying reaction times were tested for both (**tables 2 and 3**).

Table 2: Effect of different bases and reaction times on step 1 of pathway B.



Base	Equivalents of base	Reaction time	Yield
$NaHCO_3$	3	10 minutes	Traces
Na_2CO_3	10	2.5 hours	77%
K_2CO_3	10	2 hours	53%

Table 3: Effect of different bases and reaction times on step 2 of pathway B.



Base	Equivalents of base	Reaction time	Yield
DBU	5	10 minutes	N/A
DBU	5	Overnight	N/A
TMG	5	2 hours	N/A

N/A: not applicable.

5 Discussion

In this work two synthetic pathways, A and B, have been tested for the total synthesis of **compound 5**. The first route resulted from previously developed work regarding the Project II subject and was optimized during the work developed for this thesis. The second one is a novel route tested recently for the same compound.

Pathway A is comprised of 4 steps and gave a total yield of 15% using oxalyl chloride and DMF to obtain **compound 4** and 14% using thionyl chloride on the same step. This route has various challenges that were resolved in the course of this work.

Compound 2 was easily obtained within good yields, considering the addition order of the reagents. The reaction should be performed in the reverse order from the one it was described, meaning that *p*-toluenesulfonyl chloride should be added dropwise to the hydrazine instead of the contrary. This stems from when the hydrazine is added to **compound 1** there is a possibility of occurring dimer formation, which we want to avoid. However, the protocol we followed described the procedure the way it is reported in this thesis. Since the yield was good, the product was pure and the reaction time would increase, since there is considerably more volume of the chloride solution than hydrazine, procedure changes were not made.

On step 2, in a few occasions, the yield was lower than expected. It was rationalized that this happened because the filtered solid from the reaction was not properly dried, leaving water in the system. Additionally, hydrazine is a very hygroscopic compound, adsorbing the water in the system even if present in small amounts. This resulted in the absence of full precipitation of **compound 3** in the recrystallization step. When this happened, the mixture was treated with magnesium sulfate to adsorb the remaining water in the system, filtered and recrystallized again leading to loss of compound between the added steps.

The next step of the synthetic route was one of the most challenging ones. Two different methods were used to synthesize **compound 4**. The first used oxalyl chloride as a reagent and DMF as a catalyst. This reaction is very sensitive to small variations in the quantities of reagents and moisture in the reaction mixture and has not given, to this date, reproducible outcomes giving yields from 76% to 91%. The degree of purity also varies inconsistently from a white crystalline powder to a yellow oil. This was

rationalized to occur due to the use of oxalyl chloride. This reagent is difficult to remove from the reaction mixture and, when not totally removed, produces hydrochloric acid in contact with water, which accelerates the degradation of **compound 4**. One of the measures taken to optimize this reaction included the addition of the reagents in the order of their mechanism of action. This translates into adding oxalyl chloride in first place, DMF in second (to activate the oxalyl) and then **compound 3**. Other measure applied was to recrystallize **compound 4** with diethyl ether several times until the product turned from a yellow oil to a white powder. Both measures had a positive effect until some extent, but the reaction was not reproducible. The second method to obtain **compound 4** used thionyl chloride. This method had a lower yield, but it was a viable alternative to the first one, since the difference wasn't significant. This reaction requires a high temperature, since it is performed under reflux and the solvent used is toluene, which has a boiling point of 110.6 ºC. It has the advantage of being well described in the literature and it is possible to evaluate completion of reaction by organoleptic features of the reaction mixture. Another positive aspect is taking less than 2 hours until completion, instead of overnight. Nevertheless, it is a reaction that has to be performed with caution, since either a short or a prolonged time of heating can cause the product not to precipitate during the work up. This gives origin to a non-pure product and a low yield. This step is critical in pathway A because we have verified that the quality of **compound 4** affects the outcome of the final step and thus our objective, **compound 5**.

The last step of the reaction, to obtain **compound 5**, was target of optimization regarding the base. Anilines, such as the precursor of **compound 5**, are usually difficult to deprotonate due to their resonance effect. As such, the first step was to test organic bases. DIPEA and pyridine were the first choices. The reaction was performed, as aforementioned, and the crude analyzed by NMR, showing **compound 5** had not formed. Next, inorganic bases were tested. The first attempt was made with 2 equivalents of sodium carbonate and the crude was analyzed by NMR, showing traces of formed product. This led to the increase of the 2 equivalents of base to 10 since it was theorized that the problem was most likely the deprotonation of the aniline. In this attempt the results improved, having succeeded in the obtention of **compound 5** and reached a quantifiable yield of 9%. The last step into optimizing the reaction was to perform the reaction with potassium carbonate to verify if the added strength of potassium versus sodium would do any difference or if we had reached the maximum

deprotonation proportion of the precursor possible. This last attempt resulted in the obtention of the desired product, characterized by NMR, and with the significantly higher yield of 28%.

Pathway B was performed with the objective of uncovering a novel synthetic route for **compound 5** that would be more efficient timewise. This route was already described for the obtention of diazoacetates[58] and α-diazoacetamides,[120] none of which having a boronic acid with an aniline moiety as a precursor. This pathway is comprised of only two steps in a one-pot reaction, although it is necessary to synthesize the reagent N-N'-ditosylhydrazine which was not commercially available at the time.

The synthesis of N-N'-ditosylhydrazine occurred with no relevant setbacks. The only aspect worthy of mentioning is the yield obtained, which was lower than the one reported by the authors. It is though that the difference lies on the recrystallization step where the protocol describes evaporating the methanol until about half of the previous volume. In first place, this is a subjective step since we cannot measure efficiently the remaining volume of methanol once the mixture is evaporating. Moreover it is rationalized that N,N'-ditosylhydrazine is slightly soluble in methanol, contributing to the diminished yield obtained.

The first attempt at this pathway followed exactly what was described in the article,[58] and the crude was analyzed by NMR. There was no evidence that the product was successfully synthesized, although there were traces of the intermediate that is formed between the two steps. To better understand what was occurring in this reaction we decided to analyze both steps separately by isolating the intermediate.

As we had already concluded from the previous reaction that sodium and potassium carbonate were the only tested bases that could deprotonate our precursor, we proceeded to substitute the sodium bicarbonate for sodium and potassium bicarbonate in two separate reactions and then proceeded to analyze the intermediate by NMR. The analysis showed surprising results. The intermediate had formed in good yields but, contrary to pathway A, the sodium carbonate reaction had a better yield (77%) than the potassium carbonate one (53%). Normally inorganic bases are more difficult to solubilize than organic ones, hence the filtration step in all the reactions that required it. This led us to rationalize that sodium carbonate is more soluble in ACN than potassium carbonate, making the first the base of choice for further reactions.

Step 2 of this route proved to be the most challenging one, as none of the attempts made led to the obtention of the compound. Initially the reaction was performed as described in the article,[58] but quickly came to the conclusion that the α proton had lower acidity than the required one to see the reaction through and that DBU was probably not able to efficiently deprotonate it. On the next attempt instead of 10 min for the second step, the reaction was left overnight to try and reach the maximum deprotonation possible. This also did not lead to the formation of **compound 5**. Last attempt made at this route consisted in an adaptation of a procedure from an article regarding the synthesis of α-diazoacetamides,[120] where the base was switched from DBU to TMG and the reaction set to 2 hours. They are both considered very strong bases, being TMG a little more effective than DBU. This also did not change the outcome of the reaction. The most probable reason for the failure of this step of the reaction is because the α proton of the amide present in the intermediate compound is not acidic enough for the base to retrieve it.

Whilst developing this work we used real time FT-IR analysis. This technology allowed to follow reaction to obtain **compound 5**, since its band is very characteristic (2200 cm^{-1}), being inclusively a diagnostic band in the IR spectrum. It was also useful in the purification step allowing the identification of the fractions containing the product.

Regarding the envisioned applications for this product, there was not enough time to perform the appropriate experiments. Although in theory the BA moiety is capable of the recognition of molecules containing hydroxylated groups, such as serines, and the diazo moiety could do the labelling of molecules containing these motifs, we could not gather experimental data to prove that.

6 Conclusions

The work developed regarding this thesis had the primary objective of synthesizing a novel compound that combines a BA moiety and a diazo one, **compound 5**. The objective was to obtain a compound that could be used as a photoaffinity probe in order to recognize and label molecular targets of choice.

To attain this goal, two different pathways were tested. A novel synthetic route, pathway B, developed in the framework of this thesis, was tried to obtain the desired compound. This route was target of several optimization processes, mainly regarding the bases used in both steps and the reaction times. It was concluded that, on step 1, sodium carbonate was a significantly better base comparing to sodium bicarbonate and potassium carbonate. Moreover, 10 minutes were not enough for the deprotonation of the BA to occur, needing instead of 2 hours. Unfortunately, this route was not successful in reaching its goal because the intermediate formed between step 1 and 2 lacked the characteristics necessary for it to happen, the absence of sufficient acidity on the α proton.

Pathway A was the result of work developed for both the Project II subject and for this thesis and it was successful in obtaining **compound 5** within reasonable yield. Although it comprises some synthetic challenges, it is the only route, to this date, that is able to provide **compound 5**.

In future work, it would be interesting to experiment with proteins containing serines to evaluate the capability of the compound to recognize them. If results from these studies turned out favorable, this compound could have many advantageous applications. The recognition of SRRPs is an interesting application to look into since they are expressed on the surface of many Gram-positive bacteria, making for possible useful applications in clinical and analytical medicine in the future.

Bibliographic References

1. Forstinger K, Metz HJ, Koch P. Diazo Compounds and Diazo Reactions. In: Ullmann's Encyclopedia of Industrial Chemistry. 2015. p. 1–22.
2. Waldman AJ, Pechersky Y, Wang P, Wang JX, Balskus EP. The Cremeomycin Biosynthetic Gene Cluster Encodes a Pathway for Diazo Formation. ChemBioChem. 2015;16:2172–5.
3. Pechmann H V. Pechmann: Ueber. Ber Dtsch Chem Ges. 1894;146:9–12.
4. LeWINN EB. Diazomethane poisoning; report of a fatal case with autopsy. Am J Med Sci. 1949;218(5):556–62.
5. Proctor LD, Warr AJ. Development of a continuous process for the industrial generation of diazomethane. Org Process Res Dev. 2002;6(6):884–92.
6. Mix KA, Aronoff MR, Raines RT. Diazo Compounds: Versatile Tools for Chemical Biology. ACS Chem Biol. 2016;11(12):3233–44.
7. Urben PG. Reactive Chemical Hazards: Specific Chemicals. In: Bretherick's Handbook of Reactive Chemical Hazards. 1999. p. 141, 157, 182, 268, 384, 387, 404.
8. Koparde AA, Doijad RC, Magdum CS. Natural Products in Drug Discovery. In: Pharmacognosy - Medicinal Plants. 2019.
9. Köpke T, Zaleski JM. Diazo-Containing Molecular Constructs as Potential Anticancer Agents : From Diazo [b] fluorene Natural Products to Photoactivatable Diazo-Oxochlorins. Anticancer Agents Med Chem. 2008;8:292–304.
10. Nawrat CC, Moody CJ. Natural products containing a diazo group. Nat Prod Rep. 2011;28(8):1426–44.
11. Blair LM, Sperry J. Natural Products Containing a Nitrogen-Nitrogen Bond. J Nat Prod [Internet]. 2013;76(4):794–812. Available from: http://dx.doi.org/10.1021/np400124n%5Cnhttp://pubs.acs.org/doi/abs/10.1021/np400124n
12. Sugai Y, Katsuyama Y, Ohnishi Y. A nitrous acid biosynthetic pathway for diazo

group formation in bacteria. Nat Chem Biol [Internet]. 2016;12:73–5. Available from: http://dx.doi.org/10.1038/nchembio.1991

13. J. Waldman, Abraham, Ng, Tai L., Wang, Peng, Balskus EP. Heteroatom–Heteroatom Bond Formation in Natural Product Biosynthesis. 2017;117(8):5784–5863.

14. Ahluwalia GS, Grem JL, Hao Z, Cooney DA. Metabolism and action of amino acid analog anti-cancer agents. Pharmacol Ther. 1990;46(2):243–71.

15. Rahman A, Smith FP, Luc PT WP. Phase I study and clinical pharmacology of 6-diazo-5-oxo-L-norleucine (DON). Invest New Drugs. 1985;3(4):369–74.

16. Gilbert LI. Reactive Asparagine Analog with Biological Specificity. 2000;161:17–8.

17. Swain AL, Jaskolski M, Housset D, Rao JKM, Wlodawer A. Crystal structure of Escherichia coli L-asparaginase, an enzyme used in cancer therapy. Proc Natl Acad Sci U S A. 1993;90(4):1474–8.

18. Asselin BL, Lorenson MY, Whitin JC, Coppola DJ, Kende AS, Blakley RL, et al. Measurement of Serum L-Asparagine in the Presence of L-Asparaginase Requires the Presence of an L-Asparaginase Inhibitor. Cancer Res. 1991;51(24):6568–73.

19. Garbaccio RM, Parmee ER. The Impact of Chemical Probes in Drug Discovery: A Pharmaceutical Industry Perspective. Cell Chem Biol [Internet]. 2016;23(1):10–7. Available from: http://dx.doi.org/10.1016/j.chembiol.2015.11.011

20. Cheryl H Arrowsmith, James E Audia, Christopher Austin JB et al. The promise and peril of chemical probes. Nat Chem Biol. 2015;11:536–41.

21. Zorn JA, Wells JA. Turning enzymes ON with small molecules. Nat Chem Biol [Internet]. 2010;6(3):179–88. Available from: http://dx.doi.org/10.1038/nchembio.318

22. Josa-Culleré L, Wainman YA, Brindle KM, Leeper FJ. Diazo group as a new chemical reporter for bioorthogonal labelling of biomolecules. RSC Adv. 2014;4:52241–4.

23. Smith E, Collins I. Photoaffinity labeling in target and binding site identification. Future Med Chem. 2015;7(2):159–83.

24. Bayley H, Knowles JR. Photoaffinity Labeling. Methods Enzymol. 977;69–114.

25. Chowdhr V, Westheimer FH. Photoaffinity labeling of biological systems. Annu Rev Biochem. 1979;48(1):293–325.

26. Wolff L. Ueber Diazoanhydride. Justus Liebigs Ann Chem. 1902;325:129–95.

27. Kirmse W. 100 Years of the Wolff rearrangement. European J Org Chem. 2002;2193–256.

28. Meier H, Zeller K -P. The Wolff Rearrangement of α-Diazo Carbonyl Compounds. Angew Chemie Int Ed English. 1975;14(1):32–43.

29. Converse, C. A., and Richards FF. Two-stage photosensitive label for antibody combining sites. Biochemistry. 1969;8(4431−4436).

30. Xia Y, Peng L. Photoactivatable lipid probes for studying biomembranes by photoaffinity labeling. Chem Rev. 2013;113(10):7880–929.

31. Ferreira VF, Pereira LOR, De Souza MCBV, Cunha AC. Compostos α-diazo carbonílicos: Uma estratégia atraente para a síntese orgânica. Quim Nova. 2001;24(4):540–53.

32. Burtoloso ACB, Momo PB, Novais GL. Traditional and new methods for the preparation of diazocarbonyl compounds. An Acad Bras Cienc. 2018;90(1):859–93.

33. DIMROTH O. Über intramolekulare umlagerungen. Ann Chem. 1910;373:336–70.

34. Regitz M. Reaktionen aktiver methylenverbindungen mit aziden, I. Eine neue synthese für a-diazo-b-dicarbonylverbindungen aus benzolsulfonylaziden und b-diketonen. Justus Liebigs Ann Chem. 1964;676:101–9.

35. Dutra LG, Saibert C, Vicentini DS, Sá MM. Diazo transfer reaction to 1,3-dicarbonyl compounds with sulfonyl azides catalyzed by molecular sieves. J Mol Catal A Chem [Internet]. 2014;386:35–41. Available from: http://dx.doi.org/10.1016/j.molcata.2014.02.011

36. Regitz M. New Methods of Preparative Organic Chemistry. Transfer of Diazo Groups. Angew Chem Intern Ed. 1967;6(9):733–49.

37. Doyle M, Dorow R, Terpstra J, Rodenhouse R. Synthesis and catalytic Reaction of chiral N-(diazoacetyl)oxazolidones. J Org Chem. 1985;50:1663–6.

38. Danheiser RL, Miller RF, Brisbois RG, Park1 SZ. An Improved Method for the Synthesis of a-Diazo Ketones. J Org Chem. 1990;55(6):1959–64.

39. Danheiser RL, Miller RF, Brisbois RG. Detrifluoroacetylative Diazo Group Transfer: (E)-1-Diazo-4-Phenyl-3-Buten-2-One. Org Synth. 1996;73:134.

40. Taber DF, Gleave DM, Herr RJ, Moody K, Hennessy MJ. A New Method For the Construction of Alpha Diazo ketones. 1995;60(7):2283–5.

41. Taber DF, Sheth RB, Joshi P V. Simple preparation of α-diazo esters. J Org Chem. 2005;70(7):2851–4.

42. Doyle MP, Kalinin A V. Highly enantioselective intramolecular cyclopropanation reactions of N-Allylic-N-methyldiazoacetamides catalyzed by chiral dirhodium(II) carboxamidates. J Org Chem. 1996;61(6):2179–84.

43. Ouihia A, Rene L, Guilhem J, Pascard C, Bernard B. A new diazoacylating reagent: preparation, structure, and use of succinimidyl diazoacetate. J Org Chem. 1993;58(7):1641–2.

44. Fischer N, Goddard-Borger ED, Greiner R, Klapötke TM, Skelton BW, Stierstorfer J. Sensitivities of some imidazole-1-sulfonyl azide salts. J Org Chem. 2012;77(4):1760–4.

45. Ye H, Liu R, Li D, Liu Y, Yuan H, Guo W, et al. A safe and facile route to imidazole-1-sulfonyl azide as a diazotransfer reagent. Org Lett. 2013;15(1):18–21.

46. Katritzky AR, El Khatib M, Bolshakov O, Khelashvili L, Steel PJ. Benzotriazol-1-yl-sulfonyl azide for diazotransfer and preparation of azidoacylbenzotriazoles. J Org Chem. 2010;75(19):6532–9.

47. Chiara JL, Suárez JR. Synthesis of α-diazo carbonyl compounds with the shelf-stable diazo transfer reagent nonafluorobutanesulfonyl azide. Adv Synth Catal. 2011;353(4):575–9.

48. Kitamura M, Kato S, Yano M, Tashiro N, Shiratake Y, Sando M, et al. A reagent for safe and efficient diazo-transfer to primary amines: 2-azido-1,3-dimethylimidazolinium hexafluorophosphate. Org Biomol Chem. 2014;12(25):4397–406.

49. Ramachary DB, Narayana V V., Ramakumar K. Direct ionic liquid promoted organocatalyzed diazo-transfer reactions: diversity-oriented synthesis of diazo-compounds. Tetrahedron Lett. 2008;49(17):2704–9.

50. Tarrant E, O'Brien C V., Collins SG. Studies towards a greener diazo transfer methodology. RSC Adv [Internet]. 2016;6(37):31202–9. Available from: http://dx.doi.org/10.1039/C6RA03678C

51. Faisal S, Zang Q, Maity PK, Brandhofer A, Kearney PC, Reiser O, et al. Development and Application of a Recyclable High-Load Magnetic Co/C Hybrid ROMP-Derived Benzenesulfonyl Chloride Reagent and Utility of Corresponding Analogues. Org Lett. 2017;19(9):2274–7.

52. Deadman BJ, O'Mahony RM, Lynch D, Crowley DC, Collins SG, Maguire AR. Taming tosyl azide: The development of a scalable continuous diazo transfer process. Org Biomol Chem. 2016;14(13):3423–31.

53. DOYLE MP MMAYT. Modern catalytic methods for organic synthesis with diazo compounds: from cylclopropanes to ylides. New York, USA: New York: J Wiley & Sons; 1998. 652 p.

54. Holton TL, Schechter H. Advantageous Syntheses of Diazo Compounds by Oxidation of Hydrazones with Lead Tetraacetate in Basic Environments. J Org Chem. 1995;60(9):4725–9.

55. Javed MI, Brewer M. Diazo preparation via dehydrogenation of hydrazones with "activated" DMSO. Org Lett. 2007;9(9):1789–92.

56. Bamford WR, Stevens TS. The decomposition of toluene-p-sulphonylhydrazones by alkali. J Chem Soc. 1952;924:4735–40.

57. House HO, Blankley CJ. Preparation and Decomposition of Unsaturated Esters of Diazoacetic Acid. J Org Chem. 1968;33(1):53–60.

58. Torna T, Shimokawa J, Fukuyama T. N,N′-ditosylhydrazine: A convenient

reagent for facile synthesis of diazoacetates. Org Lett. 2007;9(16):3195–7.

59. Forster MO. Azotisation by Chloroamine. J Chem Soc Trans. 1915;107:260–7.

60. Hall DG. Boronic Acids: Preparation and Applications in Organic Synthesis, Medicine and Materials. Boronic Acids Prep Appl Org Synth Med Mater (Volume 1 2). 2011;1–2.

61. Yang W, Gao X, Wang B. Boronic acid compounds as potential pharmaceutical agents. Med Res Rev. 2003;23(3):346–68.

62. Liu XC, Hubbard JL, Scouten WH. Synthesis and structural investigation of two potential boronate affinity chromatography ligands catechol [2-(diisopropylamino)carbonyl]phenylboronate and catechol [2-(diethylamino)carbonyl, 4-methyl]phenylboronate. J Organomet Chem. 1995;493(1–2):91–4.

63. Lorand JP, Edwards JO. Polyol Complexes and Structure of the Benzeneboronate Ion. J Org Chem. 1959;24(6):769–74.

64. António JPM, Russo R, Carvalho CP, Cal PMSD, Gois PMP. Boronic acids as building blocks for the construction of therapeutically useful bioconjugates. Chem Soc Rev. 2019;48(13):3513–36.

65. Martínez-Aguirre MA, Yatsimirsky AK. Brønsted versus Lewis Acid Type Anion Recognition by Arylboronic Acids. J Org Chem. 2015;80(10):4985–93.

66. Frankland E, Duppa BF. Vorläufige Notiz über Boräthyl. Justus Liebigs Ann Chem. 1860;115(3):319–22.

67. EMA (European Medicines Agency). Anexo I - Resumo das Características do Medicamento - Bortezomib SUN. 2010;

68. Jinna S, Finch J. Spotlight on tavaborole for the treatment of onychomycosis. Drug Des Devel Ther. 2015;9:6185–90.

69. Zane LT, Chanda S, Jarnagin K, Nelson DB, Spelman L, Gold LFS. Crisaborole and its potential role in treating atopic dermatitis: Overview of early clinical studies. Immunotherapy. 2016;8(8):853–66.

70. Lomovskaya O, Sun D, Rubio-Aparicio D, Nelson K, Tsivkovski R, Griffith DC,

et al. Vaborbactam: Spectrum of Beta-Lactamase Inhibition and Impact of Resistance Mechanisms on Activity in Enterobacteriaceae. Antimicrob Agents Chemother. 2017;61(11):e01443-17.

71. Miyaura N, Suzuki A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. Chem Rev. 1995;95(7):2457–83.

72. Quach TD, Batey RA. Ligand- and Base-Free Copper(II)-Catalyzed C-N Bond Formation: Cross-Coupling Reactions of Organoboron Compounds with Aliphatic Amines and Anilines. Org Lett. 2003;5(23):4397–400.

73. Ishihara K, Yamamoto H. Arylboron compounds as acid catalysts in organic synthetic transformations. European J Org Chem. 1999;(3):527–38.

74. Ferrier RJ. Carbohydrate boronates. Adv Carbohydr Chem Biochem. 1978;35(C):31–80.

75. Yu H, Wang B. Arylboronic acid-facilitated selective reduction of aldehydes by tributyltin hydride. Synth Commun. 2001;31(17):2719–25.

76. Yang W, Gao X, Springsteen G, Wang B. Catechol pendant polystyrene for solid-phase synthesis. Tetrahedron Lett. 2002;43(36):6339–42.

77. Sakai M, Ueda M, Miyaura N. Rhodium-catalyzed addition of organoboronic acids to aldehydes. Angew Chemie - Int Ed. 1998;37(23):3279–81.

78. Currie GS, Drew MGB, Harwood LM, Hughes DJ, Luke RWA, Vickers RJ. Chirally templated boronic acid Mannich reaction in the synthesis of optically active α-amino acids. J Chem Soc Perkin Trans 1. 2000;(17):2982–90.

79. Niu W, Rambo B, Smith MD, Lavigne JJ. Substituent effects on the structure and supramolecular assembly of bis(dioxaborole)s. Chem Commun. 2005;541(41):5166–8.

80. Middle FA, Bannister A, Bellingham AJ, Dean PDG. Separation of glycosylated haemoglobins using immobilized phenylboronic acid. Effect of ligand concentration, column operating conditions, and comparison with ion-exchange and isoelectric-focusing. Biochem J. 1983;209(3):771–9.

81. Kitano S, Koyama Y, Kataoka K, Okano T, Sakurai Y. A novel drug delivery

system utilizing a glucose responsive polymer complex between poly (vinyl alcohol) and poly (N-vinyl-2-pyrrolidone) with a phenylboronic acid moiety. J Control Release. 1992;19(1–3):161–70.

82. Yoon J, Czarnik AW. Fluorescent chemosensors of carbohydrates. A means of chemically communicating the binding of polyols in water based on chelation-enhanced quenching. Conf Proc - Lasers Electro-Optics Soc Annu Meet. 1992;1992-Novem(8):276–7.

83. Fang G, Wang H, Bian Z, Sun J, Liu A, Fang H, et al. Recent development of boronic acid-based fluorescent sensors. RSC Adv. 2018;8(51):29400–27.

84. Smith BD, Gardiner SJ, Munro TA, Paugam MF, Riggs JA. Facilitated transport of carbohydrates, catecholamines, and amino acids through liquid and plasticized organic membranes. J Incl Phenom Mol Recognit Chem. 1998;32(2–3):121–31.

85. Fu H, Fang H, Sun J, Wang H, Liu A, Sun J, et al. Boronic Acid-based Enzyme Inhibitors: A Review of Recent Progress. Curr Med Chem. 2014;21(28):3271–80.

86. Nedunchezhian K, Aswath N, Thiruppathy M, Thirugnanamurthy S. Boron neutron capture therapy - a literature review. J Clin Diagnostic Res. 2016;10(12):ZE01–4.

87. Halo TL, Appelbaum J, Hobert EM, Balkin DM, Schepartz A. Selective recognition of protein tetraserine motifs with a cellpermeable, pro-fluorescent bis-boronic acid. 2009;131(2):438–9.

88. Sun X, Cai Y, Xu Z, Zhu D. Preparation and properties of tumor-targeting MRI contrast agent based on linear polylysine derivatives. Molecules. 2019;24(8).

89. Liu CC, Mack A V., Tsao ML, Mills JH, Hyun SL, Choe H, et al. Protein evolution with an expanded genetic code. Proc Natl Acad Sci U S A. 2008;105(46):17688–93.

90. Liu Z, He H. Synthesis and Applications of Boronate Affinity Materials: From Class Selectivity to Biomimetic Specificity. Acc Chem Res. 2017;50(9):2185–93.

91. Singh J, Petter RC, Baillie TA, Whitty A. The resurgence of covalent drugs. Nat

Rev Drug Discov. 2011;10(4):307–17.

92. Dembitsky VM, Smoum R, Al-Quntar AA, Ali HA, Pergament I, Srebnik M. Natural occurrence of boron-containing compounds in plants, algae and microorganisms. Plant Sci. 2002;163(5):931–42.

93. Baker SJ, Ding CZ, Akama T, Zhang YK, Hernandez V, Xia Y. Therapeutic potential of boron-containing compounds. Future Med Chem. 2009;1(7):1275–88.

94. Diaz DB, Yudin AK. The versatility of boron in biological target engagement. Nat Chem [Internet]. 2017;9(8):731–42. Available from: http://dx.doi.org/10.1038/nchem.2814

95. Cera E Di. Serine Proteases. Int Union Biochem Mol Biol Life. 2009;61(5):510–5.

96. Walker B, Lynas JF. Strategies for the inhibition of serine proteases. Cell Mol Life Sci. 2001;58(596–624):724–7.

97. Smoum R, Rubinstein A, Dembitsky VM, Srebnik M. Boron containing compounds as protease inhibitors. Chem Rev. 2012;112(7):4156–220.

98. Reuter H, Izaaryene M. Crystal structure of dichloro-bis(/i-pentyl)tin(IV) , Sn(CsHn)2Cl2 , tin-halide compounds - part VI. 2006;221:423–4.

99. Polgár L. The catalytic triad of serine peptidases. Cell Mol Life Sci. 2005;62(19–20):2161–72.

100. Dembitsky VM, Ali HA, Srebnik M. Recent developments in bisdiborane chemistry: B-C-B, B-C-C-B, B-C=C-B and B-C≡C-B compounds. Appl Organomet Chem. 2003;17(6–7):327–45.

101. Sun X, James TD. Glucose Sensing in Supramolecular Chemistry. Chem Rev. 2015;115(15):8001–37.

102. Guan Y, Zhang Y. Boronic acid-containing hydrogels: Synthesis and their applications. Chem Soc Rev. 2013;42(20):8106–21.

103. Hendrickson GR, Lyon LA. Bioresponsive hydrogels for sensing applications. Soft Matter. 2009;5(1):29–35.

104. Klouda L, Mikos AG. Thermoresponsive hydrogels in biomedical applications. Eur J Pharm Biopharm. 2008;68(1):34–45.

105. Danne R, Poojari C, Martinez-Seara H, Rissanen S, Lolicato F, Róg T, et al. doGlycans-Tools for Preparing Carbohydrate Structures for Atomistic Simulations of Glycoproteins, Glycolipids, and Carbohydrate Polymers for GROMACS. J Chem Inf Model. 2017;57(10):2401–6.

106. Jiang S, Escobedo JO, Kim KK, Alptürk O, Samoei GK, Fakayode SO, et al. Stereochemical and regiochemical trends in the selective detection of saccharides. J Am Chem Soc. 2006;128(37):12221–8.

107. Zhang X, Chen B, He M, Zhang Y, Peng L, Hu B. Boronic acid recognition based-gold nanoparticle-labeling strategy for the assay of sialic acid expression on cancer cell surface by inductively coupled plasma mass spectrometry. Analyst. 2016;141(4):1286–93.

108. Tran TM, Alan Y, Glass TE. A highly selective fluorescent sensor for glucosamine. Chem Commun. 2015;51(37):7915–8.

109. Wang L, Yuan L, Zeng X, Peng J, Ni Y, Er JC, et al. A Multisite-Binding Switchable Fluorescent Probe for Monitoring Mitochondrial ATP Level Fluctuation in Live Cells. Angew Chemie - Int Ed. 2016;55(5):1773–6.

110. Jung SJ, Lee JY, Kim TH, Lee DE, Jeon J, Yang SD, et al. Discovery of boronic acid-based fluorescent probes targeting amyloid-beta plaques in Alzheimer's disease. Bioorganic Med Chem Lett [Internet]. 2016;26(7):1784–8. Available from: http://dx.doi.org/10.1016/j.bmcl.2016.02.042

111. Tamura T, Hamachi I. Chemistry for Covalent Modification of Endogenous/Native Proteins: From Test Tubes to Complex Biological Systems. J Am Chem Soc. 2019;141:2782–99.

112. Jing C, Cornish VW. Chemical tags for labeling proteins inside living cells. Acc Chem Res. 2011;44(9):784–92.

113. Al-Salama ZT, Garnock-Jones KP, Scott LJ. Ixazomib: A Review in Relapsed and/or Refractory Multiple Myeloma. Target Oncol. 2017;12:535–42.

114. Aykin-Burns N, Ahmad IM, Zhu Y, Oberley LW, Spitz DR. Increased levels of

superoxide and H2O2 mediate the differential susceptibility of cancer cells versus normal cells to glucose deprivation. Biochem J. 2009;418(1):29–37.

115. Wang L, Xie S, Ma L, Chen Y, Lu W. 10-Boronic acid substituted camptothecin as prodrug of SN-38. Eur J Med Chem [Internet]. 2016;116:84–9. Available from: http://dx.doi.org/10.1016/j.ejmech.2016.03.063

116. Lee D, Park S, Bae S, Jeong D, Park M, Kang C, et al. Hydrogen peroxide-activatable antioxidant prodrug as a targeted therapeutic agent for ischemia-reperfusion injury. Sci Rep [Internet]. 2015;5(April):1–13. Available from: http://dx.doi.org/10.1038/srep16592

117. Sanchez CJ, Shivshankar P, Stol K, Trakhtenbroit S, Sullam PM, Sauer K, et al. The pneumococcal serine-rich repeat protein is an intraspecies bacterial adhesin that promotes bacterial aggregation in Vivo and in biofilms. PLoS Pathog. 2010;6(8):33–4.

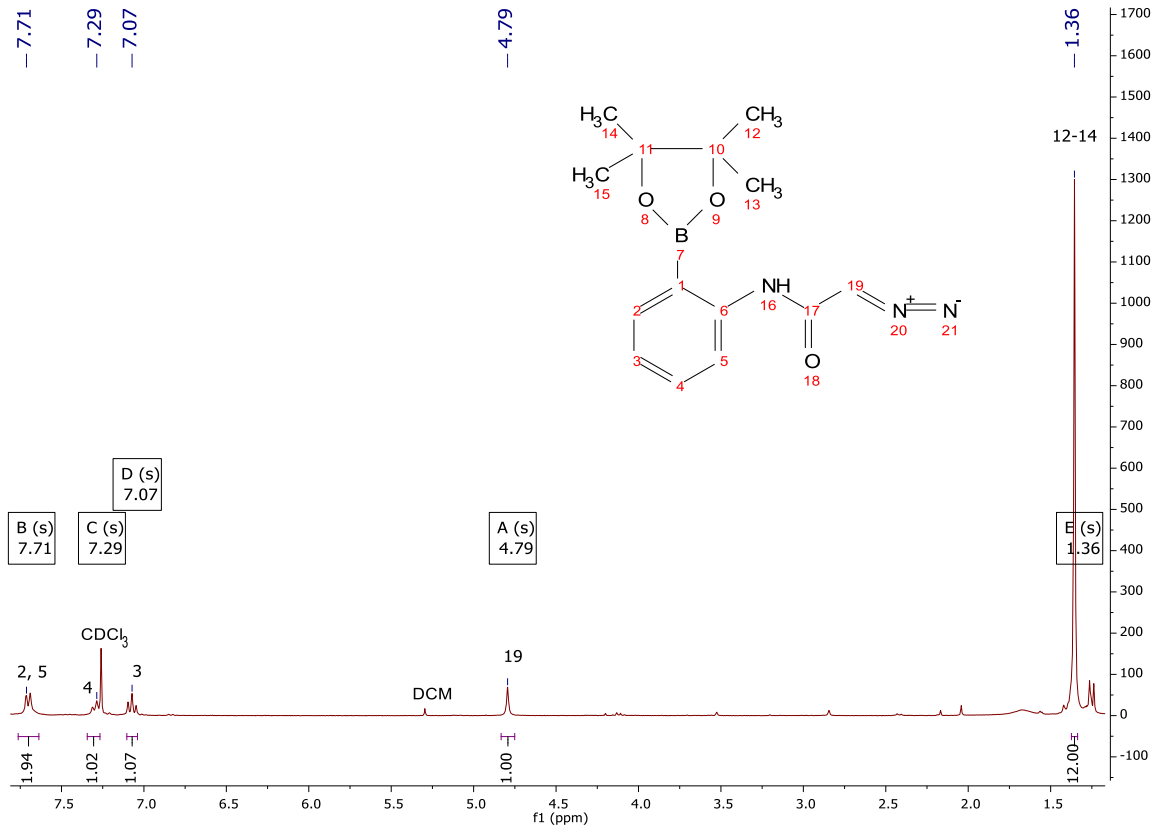
118. Friedman L, Litle RL, Reichle WR. p-toluenesulfonylhydrazyde. Org Synth. 1960;40(93).

119. Blankley CJ, Sauter FJ, House HO. Crotyl Diazoacetate. Org Synth. 1973;49:258.

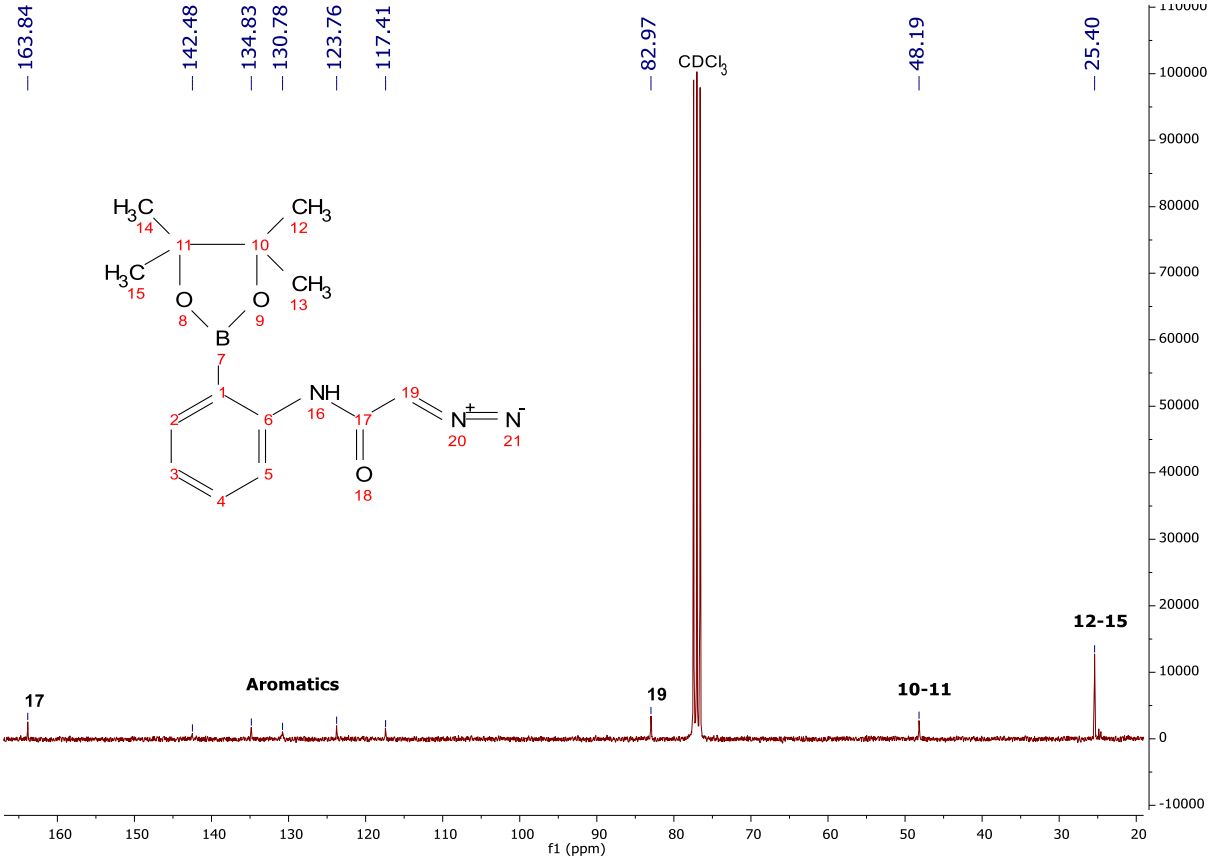
120. Kaupang Å, Bonge-Hansen T. α-Bromodiazoacetamides - a new class of diazo compounds for catalyst-free, ambient temperature intramolecular C-H insertion reactions. Beilstein J Org Chem. 2013;9:1407–13.

Annexes

A1. ^{1}H-NMR spectrum of compound 5



A2. ^{13}C-NMR spectrum of compound 5



A3. FT-IR spectrum of compound 5